An Investigation into the Mineralisation of Nitrogen and Immobilisation of Carbon and Nitrogen in Biosolids Amended Soil

Sami Ali Al-Dhumri

BSc, MSc. (Soil Science)

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School of Applied Sciences

RMIT University

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Declaration

I certify that except where due acknowledgement has been made, the work is that of the author alone; the work has not been submitted previously, in whole or in part, to qualify for any other academic award; the content of the thesis is the result of work which has been carried out since the official commencement date of the approved research program; and, any editorial work, paid or unpaid, carried out by a third party is acknowledged.

Sami Ali Al-Dhumri

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Primary Supervisor: Assoc. Prof. Nichola Porter, RMIT University

Secondary Supervisor: Assoc. Prof. Barry Meehan, RMIT University

Consultant: Assoc. Prof. Roger Wrigley, University of Melbourne

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4. Al-Omran, A.M., S. Al-Dhumri, M. Nadeem and A. El-Eter. 2009. Effect of Irrigation Regime and Emitter Depth on Yield and Water Use for Tomato. J. King Saud Univ. Agric. Sci. 21(2):43-54.

Conferences Proceedings

1. ALDHUMRI, S.A., BESHAH, F.H., PORTER, N.A & MEEHAN, B 2010. An investigation into the Transformation of Nitrogen and Phosphorus in Biosolids Amended Soil. AWA Biosolids IV Speciality Conference, Sydney, Australia.

Presentations

2. ALDHUMRI, S.A., BESHAH, F.H., PORTER, N.A, MEEHAN, B & WRIGLEY, J.R. (2013), quantifies the plant available nitrogen of biosolids. Annual Research Day, RMIT University.
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4. ALDHUMRI, S.A., BESHAH, F.H., PORTER, N.A, MEEHAN, B & WRIGLEY, J.R. An investigation into the mineralisation of nitrogen in soil Amended with biosolids under field conditions. Biosolids Workshop (14th November, 2013), linking Industry and Research. RMIT University.

Abstract

In the application of biosolids to land for agricultural purposes, the supply of plant-available nitrogen (PAN) should match the crop requirements. This ensures that the crop yield is maximised while minimising the environmental risk from over-application.

In Victoria, the amount to be applied is usually calculated according to the State EPA guidelines using the N limited biosolids application rates (NLBAR). These guidelines specify the mineralisation rates to be used in the NLBAR calculation for different types of biosolids. However, these rates have not been validated for Victorian soils and agricultural production systems. To test the veracity of these rates, this study quantified the amount of PAN for two different biosolids ((Anaerobically digested biosolids (ANDB) aerobically digested biosolids (ADB)) added to two types of soils, a sandy loam at Lara and a clay loam at the Melton Recycled Water Plant, Surbiton Park, Melton. The PAN was calculated by determining the N fertiliser equivalence of the biosolids. To achieve this, two field calibration plots were prepared, one for the biosolids and one for urea as the N fertiliser. Biosolids and urea were applied based on total N at six rates. Perennial ryegrass (*Lolium perenne*) was planted 1 day after the application of biosolids and harvested after 120 days. The calculated amount of mineralisable organic N in ANDB was estimated to be 41 % and 39% when applied to the clay loam and sandy loam soils, respectively; for ADB, it was 12 % and 9 %, respectively. These values indicate that the organic N mineralisation rates provided in the EPA Victoria guidelines (15 % for ANDB and 25 % for ADB) might not always be applicable. Also of note is that the values obtained for the each of the biosolids appear to be independent of the soil type.

The mineralisable organic N is an intrinsic property of the biosolids, which, once characterised against a fertiliser such as urea, may be able to be used to calculate its behaviour under different environmental conditions, provided the crop response to urea is known under those conditions. This was shown by the similarity in values obtained for each of the biosolids applied to two different soil types and the similarity with other Australian data. However, this needs further testing.

Prior to these calibration experiments, the rate of mineralisation of organic-N was measured in both the laboratory and in the field. In the field experiments the rate was

estimated in plots both with and without vegetation. It was found that the estimate from the laboratory experiments significantly exceed the rate estimated in the field. The addition of vegetation had only minor impact on N mineralisation: due to loss in the field which are difficult to quantify. It was concluded the calibration plots were more accurate.

Abbreviations

MRWP	Melton Recycled Water Plant at Surbiton Park site
BW	Barwon Water biosolids
WWT	Waste Water Treatment
ANDB	An aerobically digested dewatered biosolids
ADB	Aerobically digested dewatered biosolids
LA	Lara site
NBRP	National Biosolids Research Program
NLBAR	Nitrogen Limited Biosolids Application Rates
FIA	Flow injection analyser
CNR	Crop nitrogen requirements
C2 / T3	Contaminant grade 2 and treatment grade 3
C2 / T2	Contaminant grade 2 and treatment grade 2
ABN	Available biosolids nutrient
ds	Dry solids
OM	Organic matter
WHC	Water Holding Capacity
EC	Electrical Conductivity
BD	Bulk Density
M %	Moisture Content
MBC	Microbial Biomass Carbon

MBN	Microbial Biomass Nitrogen
PAN	Plant Available Nitrogen
NFE	Nitrogen Fertilizer Equivalent
DM	Dry Matter
DW	Dry Weight Basis
WD-XRF	Wave length dispersive X- ray fluorescence spectrometry
USEPA	U.S. Environmental Protection Agency
EPA	Environmental Protection Agency

Definitions

Biosolids: Organic solids produced from sewage treatment processes which have many plant nutrients such as N, P, S and K (i.e. achieve minimum standards for classification as T3 and C2 biosolids) (EPA, VIC. 2004).

C2/T3: (Contaminant grade 2 and treatment grade 3) require management during land application to ensure protection of the environment, public health and agriculture (EPA, VIC. 2004).

Class A biosolids: Have undergone treatment to the point where the concentration of pathogens is reduced to levels low enough that no additional restrictions or special handling precautions are required by Federal regulations [40 CFR* Part 503]. If the Class A biosolids meet exceptional quality requirements for metals content, they may be sold in bags and applied in the same way as other soil conditioners such as peat moss (EPA, VIC. 2004).

Class B biosolids: Have undergone treatment that has reduced but not eliminated pathogens.

By definition, Class B biosolids may contain pathogens. As a result, Federal regulations for use of Class B biosolids require additional measures to restrict public access and to limit livestock grazing for specified time periods after land application [40 CFR Part 503]. This allows time for the natural die-off of pathogens in the soil.

Contaminant grade: Grading category used to describe the quality of biosolids product based on the concentration of contaminants.

Critical nutrient concentration: The nutrient concentration in the plant or specified plant part below which the nutrient becomes deficient for optimum growth rate.

Effluent: liquid waste discharge such as residential and commercial sewage that has been treated to a quality suitable for a beneficial use.

Environmentally friendly goods: are goods produced, used or disposed of in a way that has a reduced or minimal impact on the environment.

Land disposal: Application of biosolids where beneficial use is not an objective. Disposal will usually result in application rates that exceed agronomic nutrient requirements or cause excessive contaminant accumulation in the soil.

Soil test (analysis) deficiency critical level: That concentration of an extractable nutrient element below which deficiency occurs and above which sufficiency exists.

Sustainable use: The use of nutrients in biosolids at or below the agronomic loading rate and / or use of the soil conditioning properties of biosolids. Sustainable use involves protection of human health, the environment and soil functionality.

Treatment grade: Grading category used to describe the quality of biosolids product based on a combination of defined treatment processes, microbiological criteria and stabilization to reduce vector attraction and odour generation.

National Biosolids Research Program (NBRP): A national research program coordinated by the Commonwealth Scientific and Industrial Research Organisation (CSIRO) that aims to aid revision of guidelines to maximise the benefits and minimise the risks of applying biosolids and other organic wastes to land.

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1

1 INTRODUCTION

This thesis focuses on the validation of the guideline of biosolids land application rate under Victorian climatic conditions. In Victoria, the amount to be applied is usually calculated according to the State EPA guidelines using 'Nitrogen Limited Biosolids Application Rate' (NLBAR). These guidelines specify the mineralisation rates to be used in the NLBAR calculation for different types of biosolids. However, these rates have not been validated for Victorian soils and agricultural production systems.

The NLBAR concept ensures that the biosolids applied supply enough plant available nitrogen (PAN) to meet the crop requirement. In this method, it is assumed that 15 % of the organic N from anaerobically digested biosolids and 25 % of the organic N from aerobically digested biosolids, becomes available for the crop in the first year following application (EPA, VIC ,2004). However, these mineralisation rates are based on overseas data, not on data obtained in Australia with Australian soils and climatic conditions. Therefore, these rates have not been validated for Victorian soils and agricultural production systems. This thesis describes an investigation into the amount of PAN for two different biosolids added to two types of soilsThe PAN was calculated by determining the N fertiliser equivalence of the biosolids. To achieve this, two field calibration plots were prepared, one for the biosolids and one for urea as the N fertiliser.

To investigate the influence of biosolids and soil types on the rate of organic-N mineralised, in the laboratory incubation and field incubation trials were established.

1.1 Biosolids in general context

Biosolids are stabilised sewage sludges that are obtained from various stages in wastewater treatment processes, and treated to a state so that they can be sustainably utilised for their nutrient and soil conditioning properties (EPA, VIC 2004).

Developed countries such as Australia, European countries, North America and New Zealand, have introduced regulations and guidelines to assist water authorities to safely manage the treatment, storage and utilisation of biosolids. The regulatory bodies that oversee these measures have given substantial attention to the management of biosolids in order to fully exploit their resource potential and importantly to protect human health and surrounding environments. (Tomar, 1999).

The application of biosolids for agricultural applications is by far the largest end use in most developed countries (Epstein, 2002). Biosolids contain a range of essential nutrients, trace elements, organic matter and moisture, which make them a valuable resource for farmers with the potential to partly replace chemical fertilisers (Alliance, 2007). Furthermore, application of biosolids to agricultural land has been demonstrated to improve the soil characteristics such as soil porosity, bulk density, aggregate stability and soil water holding capacity (Epstein, 2002). High quality biosolids can be produced either by composting or drying at a thermophilic temperature (> 55 °C); methods known to reduce odour, water content and destroy pathogens.

1.2 Biosolids in the Australian context

Management of biosolids after collection involves dewatering and subsequent stabilisation processes to reduce and odour and pathogens.

The annual production of biosolids in Australia approximately is 303,000 tonnes dry solids. Biosolids contain an average of 20 – 25 % solids and this corresponds to 1.2 – 1.5 million tonnes of biosolids in dewatered forms (EPA, VIC 2004). Among all the Australian states, Victoria contributes about 31 % of total biosolids produced annually, which mean that 93,466 dry tonnes of biosolids are produced each year (Table 1.1).

Table 1.1 Annual production of biosolids in Australia (2013)

States and Territories of Australia	Abbreviations	Biosolids Productions (%)
Northern Territory	NT	< 1
Tasmania	TAS	3
Australian Capital Territory	ACT	4
South Australia	SA	8
Western Australian	WA	8
Queensland	QLD	22
New South Wales	NSW	24
Victoria	VIC	31

Source: (http://www.biosolids.com.au/bs-australia.php)

The production of biosolids in Victoria is significantly higher than other States and much of this is stockpiled.

The cost of managing stockpiles has created an urgent need to find a beneficial use for these materials and the most promising option is to exploit their nutritive values by applying them to agricultural land.

1.3 Agricultural land application

Recycling biosolids and organic waste products has been the first priority for many countries over recent decades (Oliver et al., 2005, McLaughlin et al., 2007). Human / animal excreta and waste products have been used throughout history as a form of fertiliser as they were known to increase the soil fertility.

Generally, biosolids use in Australia is typical of any developed country although the percentage use of each application varies locally and from one country to other as presented in Figure 1.1. For example, it is common to burn biosolids for energy or disposal purpose in Europe and the USA as a result of the larger population and deficiency of access to appropriate agricultural land in some areas.

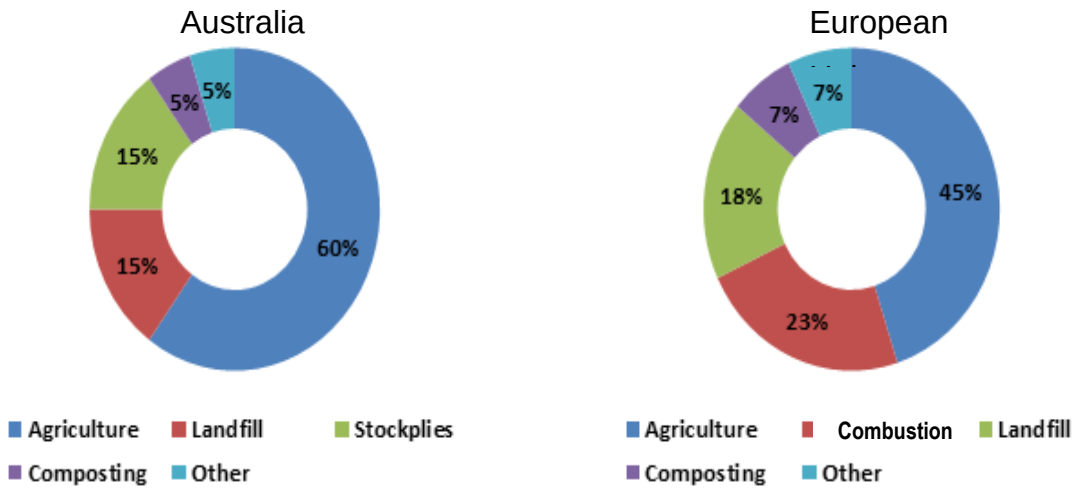

Figure 1.1. Biosolids use in Australia and European Union

Source: Australian and New Zealand Biosolids Partnership Review of Biosolids Guidelines - Summary

Australia has had a long history of carrying out large number of research investigations into the benefits and risks of utilising biosolids on agricultural land (McLaughlin et al., 2007). Since Australia is a very old continent, much of the soil nutrients have been leached away leaving most soils low in nutrients? Consequently agricultural production in Australia relies heavily on commercial fertiliser products. In recent times many farmers have trialled the use of biosolids as substitutes for commercial fertilisers. The benefits of biosolids in agricultural land applications are dependent upon the biological and organic matter present in the biosolids and the soil (O'Connor et al., 2005, Pierzynski and Gehl, 2005, Barbarick and Ippolito, 2007, McLaughlin et al., 2007, Darvodelsky, 2009, Pritchard et al., 2010, Corrêa et al., 2012, Cogger et al., 2013). The organic matter present in biosolids not only sustains plant growth but also improves soil condition, helping increase bacterial activity, enhancing soil structure and improving consistency of poor soils (Pansu and Thuriès, 2003, Jedidi et al., 2004, Moran et al., 2005, Cayuela et al., 2008, Huang and Chen, 2009). This can improve root development and access to other nutrients.

The benefits of biosolids are not only restricted to the chemical matter applied to the soil; the application of biosolids has also been observed to improve the soil's water retention, water infiltration, bulk density and porosity (Epstein, 2002, McBride et al., 2004, Sánchez-Monedero and Mondini, 2004, Marinari et al., 2010).

Melton Recycled Water Plant, and Barwon Water Treatment

In this study, two biosolids types were obtained from two different water authorities in Victoria. The first type of biosolids were anaerobically digested biosolids (ANDB) were obtained from Melton Recycling Water Plant (MRWP) (stockpiled on-site for 1 – 3 months) and were categorised as C2 / T3 (contaminant grade 2 and treatment grade 3). The EPA classifies biosolids according to contamination level for a range of metals, pesticides, and PCBs (C1 – C4), and treatments that govern the level of microbial contamination (T1 – T4). Biosolids with a classification of C1 / T1 are the highest quality products and can be used without restriction (EPA, VIC ,2004). Contaminant grade C2 requires controlled management practices. Treatment grade T2 biosolids has microbial contamination, wherein the level of contamination are well the below specified limits for microbial contamination. However, these T2 biosolids are not permitted for use on crops that are to be eaten raw. Treatment grade T3 has an additional restriction, preventing their use on areas set aside for cattle grazing. Details of these classifications and related permitted use are provided in Biosolids land application (EPA Victoria, 2004) in chapter 2.

The second type of biosolids were aerobically digested biosolids (ADB) produced at Barwon Water which had been stockpiled for 3 years and classified as C2/T2 (EPA Victoria, 2004).

1.4 Previous studies in Australia

The CSIRO Division of Soils was the first organisation to study the use of biosolids in agricultural applications and the associated environmental effects in the 1970s and these studies were undertaken over recent decades, improving our understanding of biosolids within the Australian context (AWA, 2011). During this period, accurate determination of trace metal concentrations present in soils and plants with the main focus being to estimate the uptake of potential toxic metal ions by different plants.

Apart from these earlier studies, there were several other investigations in Australia of biosolids use on land during the 1980s (Jakobsen and Willett, 1986). A major programme in biosolids research commenced in New South Wales (NSW) during the 1990s, which was coordinated by the State agriculture agency (New South Wales Agriculture, now New South Wales Department of Primary Industries). Biosolids that were produced by Sydney Water were used for these studies (Leblanc et al., 2008).

The research initiatives in NSW were driven by the concerns that guidelines for biosolids use in agriculture from other countries would not be applicable to Australian agricultural systems (Whatmuff, 1995). New South Wales has large areas of acidic light-textured soils that contain smaller organic matter content and low cation exchange capacities (CEC). However, all these characteristics increase the risk of adverse effects of metals and also increase the risk of nutrient and pathogen runoff into surface water supplies. Therefore research trials were undertaken on how to use these biosolids safely on agricultural land without any adverse effects. Furthermore, this programme included studies on the accumulation of applied metals by grazing livestock as well as the survival of various parasitic and pathogenic organisms (Eamens et al., 2006). As a result of this research program, New South Wales was the first State in Australia to produce biosolids guidelines (NSW EPA, 1997). Other States in Australia essentially used these guidelines as a template to produce their own State-based guidelines with minor differences, and current Australian national biosolids guidelines are based on the NSW guidelines.

The application rate of biosolids are dependent on total N content, the crop requirements of N, available N and the mineralisable N present in the biosolids. Available N is considered to be all nitrate-N and one fifth of the ammonium-N (to account for volatilisation losses). Mineralisable N is expected to be between10 - 25 % of the organic N in the first year after application depending on treatment process (NSW EPA, 1997).

The CSIRO Centre for Environmental Contaminants Research established the National Biosolids Research Program (NBRP) (http://www.clw.csiro.au/cecr/) in 2002 in order to coordinate the research relating to the benefits and risks of using biosolids in agriculture. The NBRP is a group of seven Australian research agencies, which are supported by several metropolitan, regional water authorities, and State-owned environmental and natural resource management agencies. NBRP initiated field trials that focus on the Cd, Cu and Zn contamination, which were considered as potential environmental risks. Cadmium contamination in the agricultural soils has become a major concern due to the toxic effects of cadmium and it increased the public awareness and concern for food and land quality. Residual cadmium present in foods is regularly analysed by agricultural and health agencies both nationally and globally (McLaughlin et al., 2007, Pritchard et al., 2010). Since Cu and Zn are known

to affect the soil microbial health when they are present in high concentrations, these two metals were also selected for metal contamination studies (Giller et al., 1998). Subsequently, the research focus was shifted to examine the potential risks from pathogens, pharmaceuticals, endocrine disrupting compounds and personal care products (NBRP, 2007). Apart from these potential risks, the benefits of nutrients and organic matter present in biosolids on crop growth are also being evaluated, with various cropping systems around Australia (NBRP, 2007).

Although NBRP was not focused on the N and P nutrients present in the biosolids, some experiments were conducted in the spring/summer growing seasons in Southern Queensland and indicated that the mineralisation of biosolids were found to be three times higher than the estimated rates of 15 and 25 % for anaerobically and aerobically digested sewage sludge (NBRP, 2007).

These results from the Queensland field trials were further validated by doing laboratory incubation studies and it was observed that loss from denitrification was greater than losses from volatilisation. Moreover, 30 % of the N present in biosolids was mineralised during the 3 months period after the biosolids application on the land.

NBRP also made suggestions regarding P management on biosolids amended land, which states that if biosolids are applied based only on crop N requirement, the excess P may accumulate and may result in a substantial off-site P movement in run-off or leachate contributing to eutrophication of aquifers. Therefore, the efficient management of N and P must take into account a range of soil properties and the various biosolids obtained from different waste water treatment plants, as recommended by NBRP (NBRP, 2007).

In Victoria

Victorian wastewater treatment plants face a major technical challenge to manage the large 66,700 dry tonnes of biosolids produced annually (EPA Victoria, 2004). There is not enough space to expand the stockpiles, and there are restrictions on landfill places, which demand the sustainable management of biosolids.

Between 2003 and 2006, the Victorian Department of Primary Industries, in collaboration with the CSIRO, studied the plant, soil and microbial measures from five biosolids and two metal salts as part of NBRP (NBRP, 2007).

The biosolids trials were conducted at Dutson Downs (cropping), Dookie (cropping), Melton (cropping), Pakenham (pasture) and Mildura (grape vines) which were scheduled to determine the effects of biosolids derived nutrients and metals on the plant/soil system for a range of crops and soil types.

In each trial site, these biosolids were applied at six different application rates and the results were compared with mineral fertiliser and control treatments. The application rates of these biosolids were estimated using the annual crop N requirement and the N percentage of the biosolids (NLBAR). Samples from the soil and plant were collected and analysed to assess the crop production, concentrations of nutrients and metals accumulated. On two occasions, samples of soil were collected after the application of biosolids and at the harvest time. Crops were sampled during mid-tillering (8 - 12 week growth stage) and at harvest. Pasture (ryegrass / clover) was sampled at seven targeted times over three years and grapes were sampled annually from the vines.

Metal contamination studies were carried out at Dookie and Dutson Downs and the main objective was to investigate the response of plants and biota to three metals commonly found in biosolids: cadmium, copper and zinc. Here again, samples of soil collected after the application of metal salts and after the harvest. In all these trials, plant and soil samples were analysed to determine the yield, metal concentrations and microbial activity (NBRP, 2007).

All these trials showed that increased biosolids application rates resulted in an increase in P and N concentrations in the soil, as well as making the soil pH more neutral (McLaughlin et al., 2007). However, these effects decreased over time. Overall, grain yield in biosolids amended soil was lower than the grain yield in the control experiment. This may have been due to a lack of soil moisture in the grain development stage.

1.5 Significance of the research work

Most of the N in biosolids is in the organic form, which is not immediately available to plants; however, over time, organic N is converted to mineral N (NO_3-N and NH_4-N) via decomposition by microorganisms (mineralisation and nitrification). The mineralised N is then available to the crop. Ideally, the application rates for biosolids should match the crop requirement for N, thus minimising the potential for

environmental risk through losses of N. These losses can occur through leaching (as nitrate) and volatilisation (as ammonia or nitrous oxide). If there is insufficient N available, crop production will be compromised (Smith and Durham, 2002, Morris et al., 2003, Corrêa(1), 2004, Rigby et al., 2010). Therefore, accurate assessment of the mineralisation rates of organic N in biosolids is necessary for their safe and efficient application to land.

In Victoria, the application rate for biosolids is calculated according to the 'nitrogen limited biosolids application rate' (NLBAR). The NLBAR concept is designed to ensure that the plant-available N (PAN) supplied meets the crop requirement without leaving a surplus of N. In this method, for the first year following application, the State EPA guidelines recommend using mineralisation rates of 15 % of the organic N from anaerobically digested biosolids (ANDB) and 25 % of the organic N from aerobically digested biosolids (ADB) (EPA Victoria, 2004). However, data from the limited number of field trials reported in Australia with Australian soils, climatic conditions and agronomic systems do not support these application rates (Eldridge et al., 2008, Pu et al., 2008, Rigby et al., 2010)(Pu et al. 2008; Eldridge et al. 2008; Rigby et al. 2010).

Research in Australia and elsewhere has demonstrated that the mineralisation of organic N in soils amended with biosolids depends on environmental and climatic conditions, including soil type, moisture, pH, temperature, C/N ratio, and type of biosolids (Tester et al., 1977, Honeycutt et al., 1991, Smith et al., 1998a, Smith et al., 1998b, Sierra et al., 2001a, Rahman and Rashid, 2002, Morris et al., 2003, Wennman and Kätterer, 2006, Pu et al., 2012). However, there is a research gap regarding the quantification of PAN from different biosolids applied to different soil types in Victoria.

1.6 Hypothesis and Research objectives

The hypotheses tested were that the mineralisation rates currently adopted by EPA Victoria for the calculation of NLBAR are different from the actual mineralisation rates for ANDB and ADB on two sites in Victoria and that result from laboratory experiments will give different results from field trials.

The hypothesis was tested by calculating the mineralisation rates using the calibration method of Barbarick and Ippolito (2000), and determining the estimate of

error from the standard error of the slopes of the two calibration curves. Field trials were compared with laboratory trials to test the second hypothesis.

To address the subjects identified above, the objectives of the research described in this thesis were to:

- examine the influence of two fertilisers and biosolids amended on a clay loam (Red Sodosol) and sandy loam soils (Brown Sodosol) on mineral N dynamics under optimum moisture and temperature conditions in the laboratory controlled environment
- investigate the microbial biomass C and N dynamics in two conventional fertilisers and biosolids amended soils under laboratory controlled conditions
- estimate the mineralisable N fractions in two fertilisers and biosolids amended soils during 91 days of incubation period
- investigate the effect of soil type, biosolids type and ryegrass on N mineralisation and immobilisation of C and N
- evaluate the effect of environmental factors such soil temperature and soil moisture on N mineralisation and immobilisation of C and N
- calculate the mineralisable portion of the organic N in the two biosolids types applied on to two soil types during 107 days of field period.
- quantify the amount of plant available nitrogen (PAN) in two soil types, a clay loam and a sandy loam, amended with two different types of biosolids, under different field conditions, in Victoria, Australia.

1.7 Thesis arrangement

The first chapter has presented a general introduction and outline of the research objectives, Chapter 2 provides a literature review of the literature available relevant to the N transformation process in soils receiving biosolids The review section highlights the effect of using various types of biosolids types relative to conventional fertiliser on the N availability from biosolids where applied to different soil types. It also describes the dynamics of N in soils treated with different biosolids under laboratory incubation and field conditions. A general description of the two sites and the details of the analytical techniques employed to characterise the two biosolids amended soil samples as provided in chapter 3. Chapter 4 describes the investigation of the dynamics of N and soil microbial biomass C and N in two soils

amended with two types of biosolids under controlled temperature and moisture conditions for 91 days. Similar experiment established under field conditions without and with vegetation of perennial ryegrass is described in Chapter 5 and 6. The Plant available N (PAN) from the two biosolids type's amended two different soil types is presented in Chapter 7 using the calibration method rather than mass balance method.

2

2 LITERATURE REVIEW

2.1 Introduction

This chapter covers the literature related to the land application of biosolids, which are obtained by stabilising sewage sludges produced during biological treatment of wastewater. Biosolids contain significant amounts of macro and micro nutrients as well as organic matter and therefore have potential as alternatives to inorganic fertilisers in agricultural production.

However, biosolids, by their very nature can have elevated levels of pathogens and other contaminants and so require treatment before they can be land applied. This chapter describes the land application guidelines in place in Australia and a number of other countries to protect receiving soil environments. In addition, the chapter reviews crop responses to land applied biosolids from different sources and the nutrient dynamics in receiving soils compared to conventional fertiliser products. As the present project has a Victorian focus special attention will be given to the issues relating to the management and land application of biosolids in Australia including wastewater treatment processes where they are generated, biosolids treatment processes and current research on the utilisation of biosolids in agricultural systems.

2.2 Wastewater treatment processes

Wastewater treatment processes generally involve multi-step procedures as shown in Figure 2.1. The initial step in this process is to remove coarse solids and grit from the wastewater with the help of screens and other filtering equipment. This is usually followed by gravity sedimentation to remove the solids that settle to the bottom of a primary clarifier. After the removal of the coarse suspended particulate matter from

the wastewater, secondary treatment of the wastewater involves a biological process to degrade dissolved and suspended organic material producing carbon dioxide, water and energy for the microorganisms as well as a sludge usually termed as secondary sludge, waste activated sludge, trickling filter humus or biological sludge (UNEP, 2002).

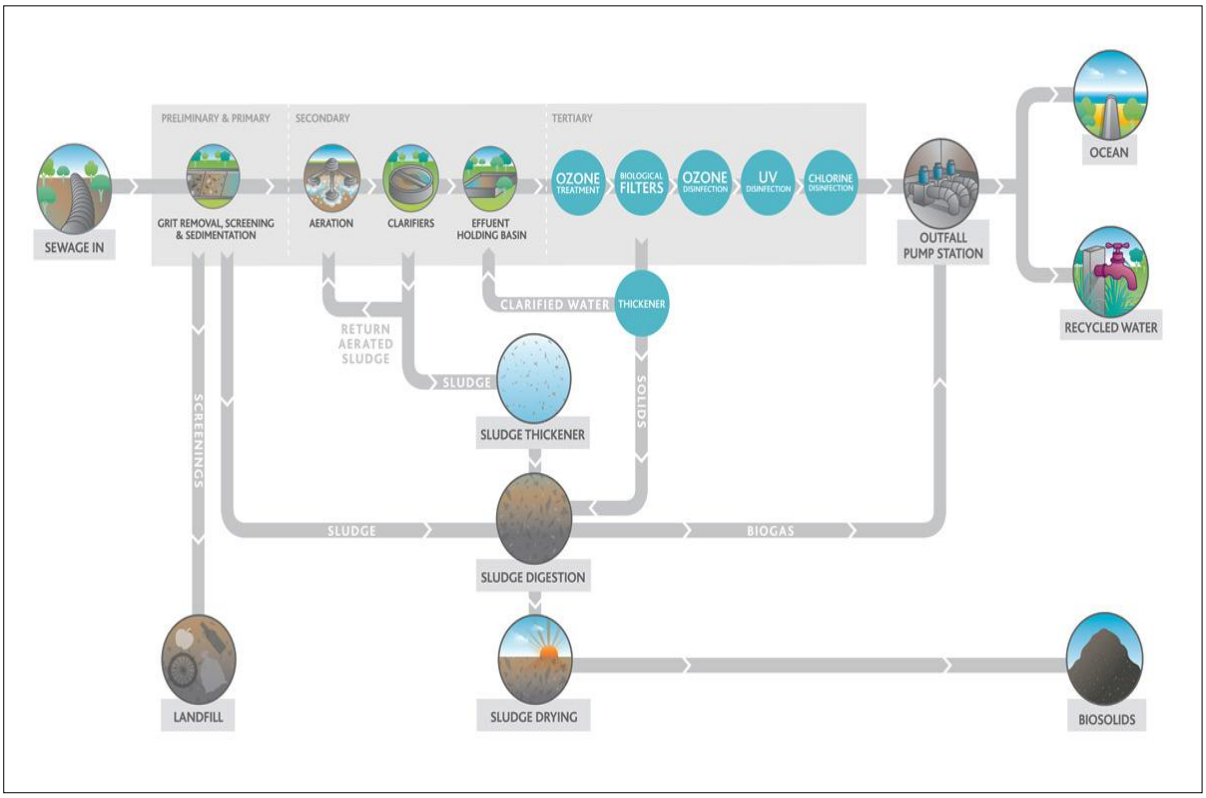

Figure 2.1. General outline of wastewater treatment process and biosolids productions

Source: http://www.melbournewater.com.au/images/sewerage/etp_process_large.jpg

Sludges collected at various stages of the wastewater treatment process are then combined and sent to a digester (operating under anaerobic or aerobic conditions) and subsequently dewatered to produce a biosolids product ready for further treatment such as stockpiling and ageing on drying pans and/or composting with various other organic waste streams such as municipal and industrial green wastes.

2.2.1 Anaerobic digestion

The anaerobic digestion is one of the established processes for viable processing of residues and wastes, suitable for agro-food industry (Taricska et al., 2007). This process can be used both for the treatment of biodegradable wastes and

manufacturing value-added products. In this process, complex organic matter is degraded by microorganisms under anaerobic conditions and simpler substances formed as a result of this anaerobic digestion. In the absence of oxygen, these microorganisms digest the organic matter to produce solid residues and gaseous by-products such as methane and carbon dioxide. The feed wastes are first transferred into a digester where the digestion takes place. However the design and type of digester depends upon the nature of feed waste, climate and other physical conditions.

In general, during the wastewater treatment processes, the anaerobic digestions are carried out in two temperature ranges (Taricska et al., 2007):

Mesophilic Anaerobic Digestion (MAD): In this regime, the temperature of anaerobic digestion is usually carried out between 30 to 37.7°C. Therefore the optimum operating temperature is usually maintained at 35 °C in the majority of wastewater treatment plants (Taricska et al., 2007).

Thermophilic Anaerobic Digestion (TAD): During this digestion regime, the operating temperature ranges from 49 to 57°C, while the optimum thermophilic temperature is maintained at t 55°C (Dohányos and Zábranská, 2001, Taricska et al., 2007). TAD is found to be advantageous over conventional MAD for a number of reasons. In this method, the rate of methane production, conversion of organic matter into biogas, pathogen inactivation is significantly increased. At the same time, fluid viscosity and biomass formation is considerably decreased (Dohányos and Zábranská, 2001).

2.2.2 Aerobic digestion

Aerobic digestion methods can be carried out in many different ways. (Shammas and Wang, 2007):

In the conventional aerobic digestion method, either air or oxygen used as an oxidant

In the case of auto thermal aerobic digestion, air (ATAD-Air) or oxygen (ATAD-Oxygen) is used to aerate/oxidise the sludge, but runs in the thermophilic temperature range (> 45°C). Aerobic digestion is an exothermic process due to the breakdown of organic and other cellular material, which provides the heat to increase the temperature. Therefore, this process is called an auto-thermal aerobic digestion.

As a result of thermophilic temperature range, the required retention time is considerably reduced for the given amount of reduction in solids (Shammas and Wang, 2007).

Auto thermal aerobic digestion using vertical shaft reactor:

This method is the modification of the existing ATAD-Air and ATAD-Oxygen processes by carry out the digestion in a vertical shaft reactor. This reactor is specifically designed for high oxygen transfer efficiency, short retention times (35 - 45 % volatile solids can be reduced in 3-6 days), lower power requirements and a small foot print for construction. A typical vertical shaft reactor is 350 ft in length and 2.5 - 10 ft in width. (Shammas and Wang, 2007).

Cryophilic Aerobic Digestion

This method allows the aerobic digestion process to occur at relatively in lower temperature ranges (< 20 °C) and is a suitable method for the waste water treatment plants located in countries in cold climates. Since this process occurs at low temperatures (5 – 20°C), it requires longer solid retention times and in some cases at least 250 – 300 days are required to remove an acceptable percentage of volatile solids (Shammas and Wang, 2007).

The principles and basic operating methods of all of the afore-mentioned aerobic digestion processes are almost the same. All these processes falls under the category of “suspended-growth biological treatment” process for the stabilisation of biosolids produced at wastewater treatment plants (Shammas and Wang, 2007). Overall, microorganisms oxidize the biodegradable matter using air or oxygen as oxidant during the process of aerobic digestion and results in the formation of microbial cellular material. The following chemical equations represent the afore-mentioned two-step oxidation reaction (Shammas and Wang, 2007):

Organic matter + O_2 → Cellular material + CO_2 + H_2O Eqn. 1

Cellular material + O_2 → Digested sludge + CO_2 + H_2O Eqn. 2

The second step depicted in chemical equation 2 is termed as endogenous respiration because this phenomenon is common in microorganisms; when microorganisms do not find any nutrients/food available to them, in order to get

energy they start digesting their own protoplasm. The cellular tissue is aerobically oxidised into ammonia, water, carbon dioxide, or nitrates during endogenous respiration, while the energy released during this process is utilised to form new cellular material and engage in further aerobic digestion (Shammas and Wang, 2007).

2.3 Stabilisation of biosolids and dewatering techniques

The total and mineralised N content present in the biosolids reported in the literature spans a wide range due to the combination of several factors including the sewage sludge stabilisation methods, process of wastewater treatment process, and the method of dewatering. Stabilisation of sludge can be done by chemical, physical and biological processes. Anaerobic, aerobic digestion or composting processes are the common biological processes, while lime treatment and pasteurisation or thermal drying is the common chemical and physical processes respectively (NSW EPA, 2000, DEP, 2002, EPA, VIC 2004). The total and mineral N concentration present in biosolids is completely dependent upon the nature of organic matter decomposition and the other chemical processes during the stabilisation. This is the major reason for the range of total and mineralisable N percentages that are usually reported in the literature. The mean total N concentration estimated from the raw, aerobically digested, mesophilic/thermophilic anaerobically digested and thermally dried biosolids is > 4 %. However, the mean total N content present in the biosolids obtained from composting or lime treatment are 2 % and 3.2 % respectively (Figure 2.2). The main reason for that the decrease in N is due to the addition of bulking agents during composting (at approximately 30 %) such as woodchips, garden waste or lime or lime treatment of sewage sludge (Christie et al., 2001, Cogger et al., 2004). Mineral N content obtained from the mesophilic anaerobically digested sludge (equivalent to 13.5 % of the total N content) is generally higher than the mineral N content of raw sludge (mean of 4.7 % total) due to the organic N losses during digestion. Lime treatment, thermal drying or composting, increase the loss of ammonia as vapour due to the increase mechanical agitation, temperature or pH and the obtained biosolids have the average mineral N content of 3.4 – 5.2 % total N. Biosolids obtained after thermophilic anaerobic/aerobically digested generally have the highest mineral N content of 18.6% of the total N content. However,

biosolids with highest mineral N content are quite rare and there is not much reported literature about these types of biosolids.

The total and mineralisable N content and the stabilisation process dictate the application of the biosolids, pathogens and contaminants. Thermal drying, lime treatment or composting resulted biosolids are suitable for unrestricted use, if they meet the specific guidelines for use of biosolids such as time of stabilisation, temperature or pH (NSW EPA, 2000, DEP, 2002, EPA, VIC 2004). The kinds of biosolids obtained after mesophilic anaerobic digestion can be used are suitable for restricted use in forestry, mine-site rehabilitation and agricultural applications with restrictions.

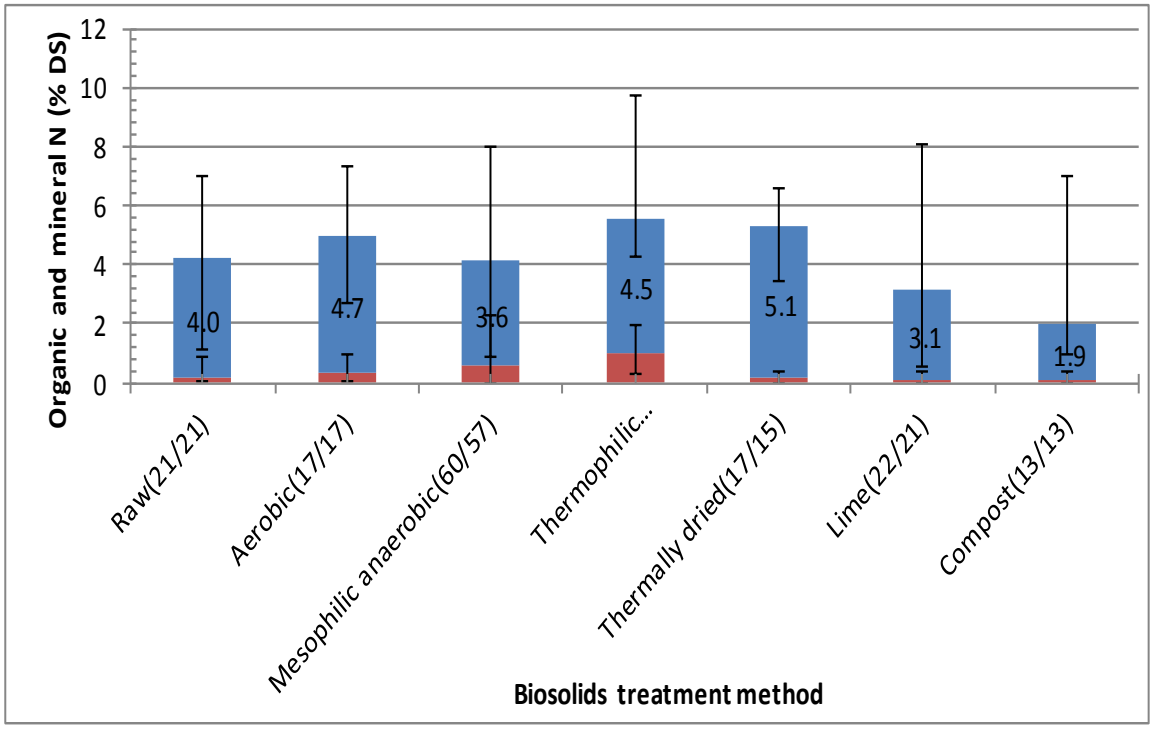

Figure 2.2. Mean organic N (% DS) (blue) and mineral N (% DS) (red) in various biosolids types (references in Appendix A, Table A1); sample number for total N/mineral N in parenthesis and the bars show the range of organic N/ mineral N concentrations.

Source: (Land application biosolids, Nitrogen and phosphorus management. final report as per Milestone 2, RMIT with the support of Smart Water Fund, Project 612-001)

In Australia, nearly 75 % of biosolids obtained after standard stabilisation are suitable for use under the biosolids guidelines. Stabilised biosolids are further classified in to Grade A and B. The stabilisation processes that generally take a long

period of time for pathogens to be completely destroyed and the biosolids obtained by this method fall under the category of Grade A biosolids. Grade A biosolids produced in a relatively high proportion (41%) in Australia; 36 % is produced as Grade B. The proportion of the un-stabilised biosolids is of greatest concern to the industry is Grade C, which is shown in the (Figure 2.3). These materials are associated with strong odour and should not recommend for any applications. The significant fraction of the unspecified biosolids is also designated to fall under Grade C.

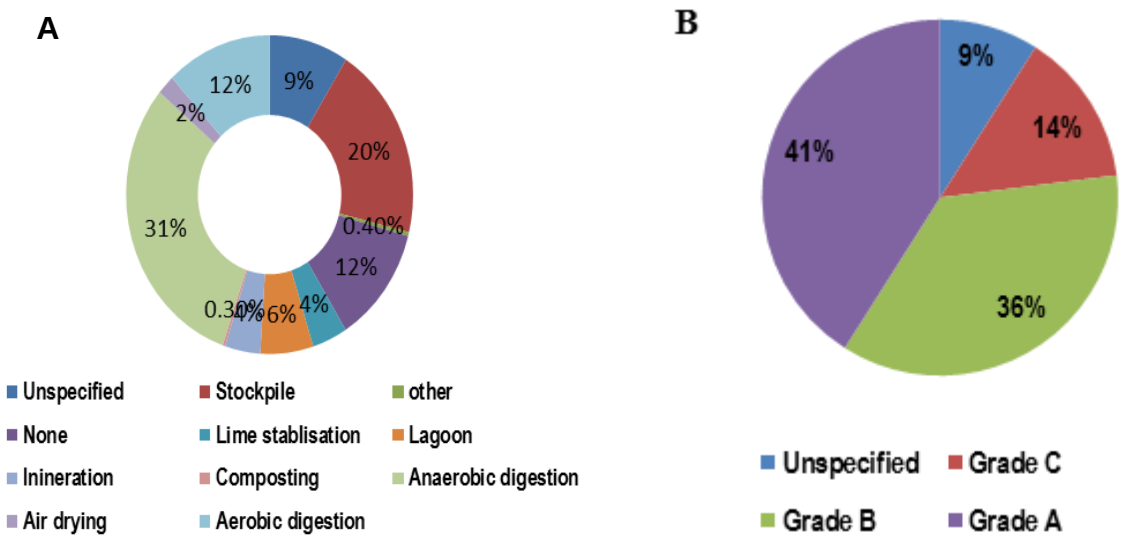

Figure 2.3. Stabilisation processes (A) and Biosolids stabilisation grade (B) in Australia

Sours: Department of Sustainability, Environment, Water, Population and Communities, BIOSOLIDS SNAPSHOT, (2011) (http://www.environment.gov.au/resource/biosolids-snapshot)

2.4 Biosolids classification

In Victoria, concentrations of contaminants present in the biosolids and the microbial quality after the treatment process are the two major parameters by which the biosolids are classified. Classification of biosolids is based on the concentrations of contaminants present in the biosolids and the microbial quality after passing the treatment processes. Contaminant grade (C1 or C2) and treatment grade (T1, T2 and T3) are the two major classification of biosolids based on the factors described above. The microbiological treatment technology employed during stabilization and the processes used to reduce bacterial regrowth, vector attraction and odour (EPA

Victoria, 2004) have to be taken into consideration for classification. The categorisation of biosolids based on the treatment process, there and chemical grade and their allowable end use send applications are also summarized summarised in Table 2.1

The classifications of biosolids can be made based on their unrestricted and restricted agricultural land applications. Biosolids that have restricted applications (e.g. C2 / T1, C1 / T1, and C2 / T3) require appropriate management during land application in order to ensure avoid and other unwanted side effects in protection of the environment, the public health, environmental conditions and agriculture.

Contaminant grade C1 biosolids are the best quality biosolids containing very low levels of contaminants and their agricultural land applications don't require any specific management control. The C2 limit provides the maximum allowed concentration of contaminants after treatment and above which contaminant levels are regarded as excessive (EPA Victoria, 2004).

Table 2.1 Classification of biosolids based on treatment and chemical grade and their permissible end uses

Treatment grade	Chemical Grade	"Unrestricted"	"Restricted uses"					
			Agricultural Uses				Non-Agricultural uses	
			Human food crops consumed raw in direct contact with biosolids	Dairy and cattle grazing? fodder (also poultry), human food crops consumed raw bot no in direct contact	Processed food crops	Sheep grazing and fodder (also horses, goats), on food crops, woodlots	Landscaping (unrestricted public access)	Landscaping (restricted public access), forestry, land rehabilitation
T1	C1	✓	✓	✓	✓	✓	✓	✓
T2	C1	X	X	✓	✓	✓	✓	✓
T3	C1	X	` X	X	✓	✓	X	✓
T1	C2	X	✓	✓	✓	✓	✓	✓
T2	C2	X	X	✓	✓	✓	✓	✓
T3	C2	X	X	X	✓	✓	X	✓

✓ Indicates to the biosolids grade that will be acceptable for the end use. Biosolids grades less than T1C1 will be focus to management controls.

X refers to the biosolids of this class are not satisfactory for the end use.

Adapted from Guidelines for Environmental Management, Biosolids Land Application, (EPA Victoria, 2004)

2.5 Agricultural Land Application of Biosolids

In Australia, a recent survey (2013) estimated that 330,000 tonnes of biosolids produced annually (http://www.biosolids.com.au/bs-australia.php) and state wise productions of biosolids are given in Figure 2.4. The average solid content of these biosolids is estimated to be 20 – 25 % and this corresponds to 1.2 – 1.5 million tonnes of biosolids in dewatered form.

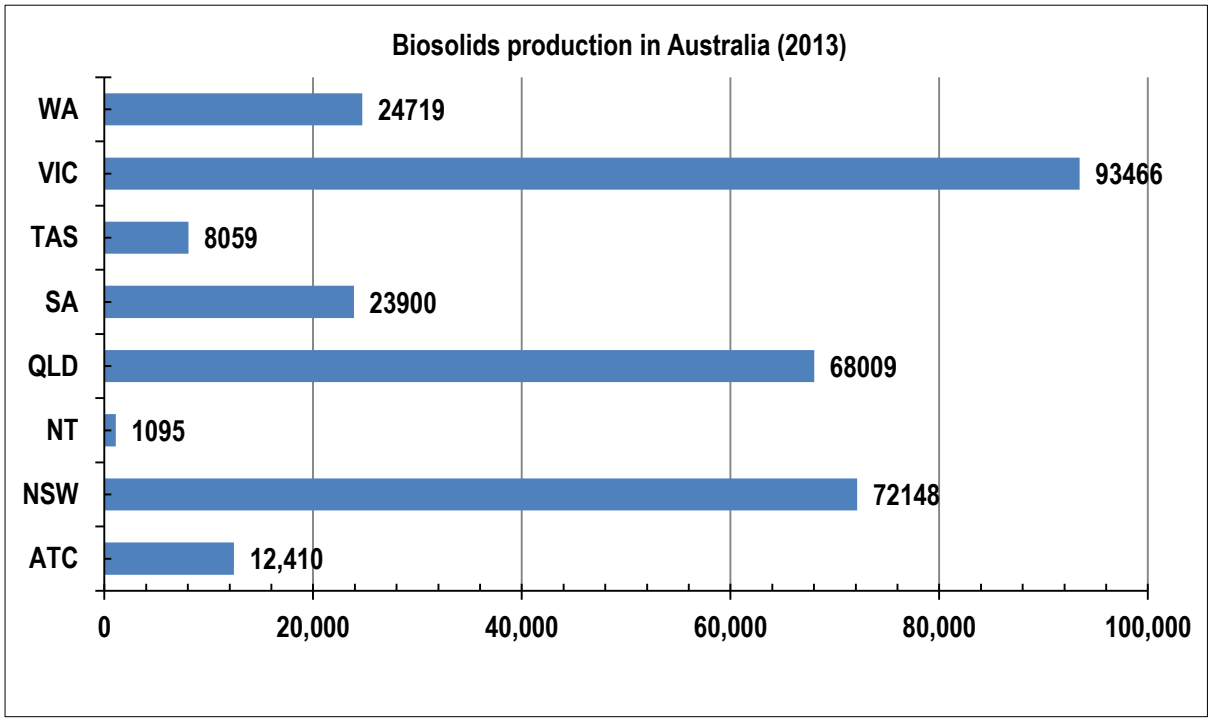

Figure 2.4. Total biosolids production (dry basis tonn per year) in all Australian States (2013), WA refers to Western Australia, Victoria (VIC), Tasmaina (TAS), South Australia (SA), Queensland (QLD), Northern Territory (NT) and New South Wales (NSW)

Source: Department of Sustainability, Environment, Water, Population and Communities, BIOSOLIDS SNAPSHOT, (2011) (http://www.environment.gov.au/resource/biosolids-snapshot)

Overall two thirds of biosolids produced in Australia are utilised as soil conditioners or fertilisers in land application. Agricultural utilisation of these materials contributes to the largest end use in Australia, followed by their use in composting. However, biosolids are also stockpiled. Stockpiling biosolids is a problem because of the land required and the costs associated with their management (http://www.environment.gov.au/resource/biosolids-snapshot).

These stockpiles also need to be maintained under anaerobic conditions and as a consequence they emit very high greenhouse gas emissions in the form of methane. Therefore, stockpiling of biosolids is not a suitable option (Figure 2.5).

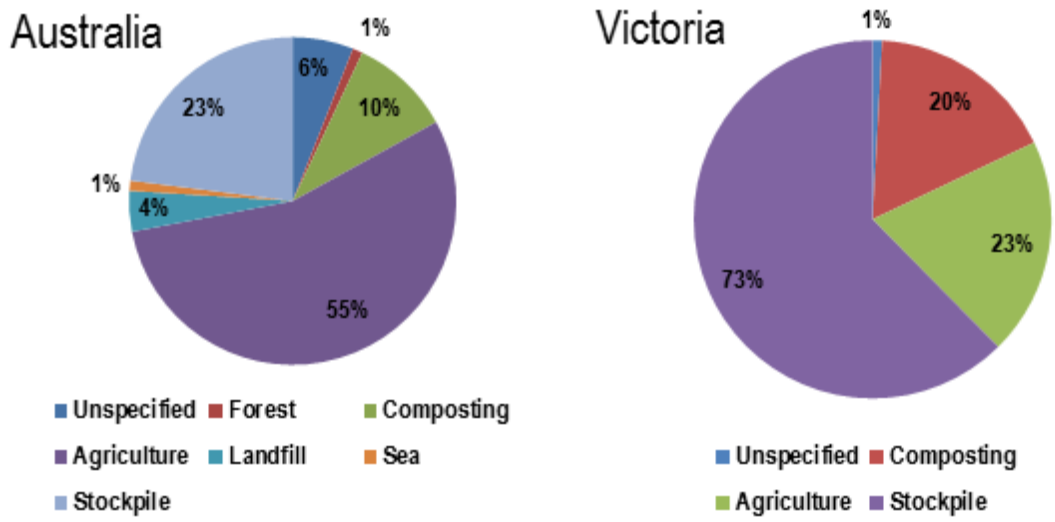

Figure 2.5. Completion of using biosolids in Australia and Victoria (2011)

Source: Department of Sustainability, Environment, Water, Population and Communities, BIOSOLIDS SNAPSHOT, (2011) (http://www.environment.gov.au/resource/biosolids-snapshot)

2.6 The impacts of biosolids on receiving soils

Even though the amount and nature of nutrients determine their use for land application, biosolids are further classified based on their physical, chemical, and biological characteristics. These characteristics affect the method of agricultural application, as well as the soil's physical and chemical properties, which in turn have an overall effect on plant growth. The availability of trace elements is significantly influenced by biosolids characteristics and treatments. Solid content, particle size and moisture content are the major physical characteristics of the biosolids, pathogen also determine land application of biosolids. Solid content, particle size and moisture content determine biosolids management on lands.

Chemical properties of the biosolids play a key role in plant growth as well as it influences the soil's chemical, biological and physical characteristics. The important chemical characteristics of a typical biosolids include the pH, organic chemicals, essential and non-essential trace elements to humans/animals, plant micro and

macro nutrients. Quality of wastewater, use of chemicals (ferric chloride, polymers,etc) method of stabilisation (e.g. lime treatment) and the extent of treatment (primary, secondary, tertiary), are the major factors that control the chemical characteristics of the biosolids (Epstein, 2002). In addition to the inorganic minerals, application of biosolids increases the organic matter content of the soil. It depends on soil's native organic matter content and bioolids application rate. The addition of organic matter improves the soil's physical properties, in particular the soil structure, soil moisture retention, and the cation exchange capacity of the soil (Epstein, 2002). However, the presence of organic matter and its beneficial use in improving the soil structure can take significant time and requires repeated applications to soil. The quantity and percentage amount of solids in biosolids dictates the trace elements presence and the amount of plant nutrients. In general, most of the biosolids both in the dewatered form or semisolid form are usually spread on the topsoil and subsequently ploughed into the soil.

Biosolids contain different types of microorganisms come from wastewater or sewage, which originally come from the stabilisation process. The majority of them can be beneficial whereas a few of them may be harmful to humans, animals and plants. These microorganisms are responsible for the biological characteristics of these biosolids, which have a strong influence on soil's microbial population, decomposition of organic matter in soil, the environment and human health (Epstein, 2002).

2.7 Biosolids Nitrogen Transformation and Loses

N present in the soil is broadly classified into organic and inorganic forms of N and both forms undergo many chemical transformations (Figure 2.6). Most of these transformations are usually mediated by different types of microorganisms present in the soil, the nature of the soil and temperature. Therefore it is important to understand the commonly occurring N transformation processes in the soil that will determine plant uptake of N. N transformation involves many processes as presented in Table 2.2.

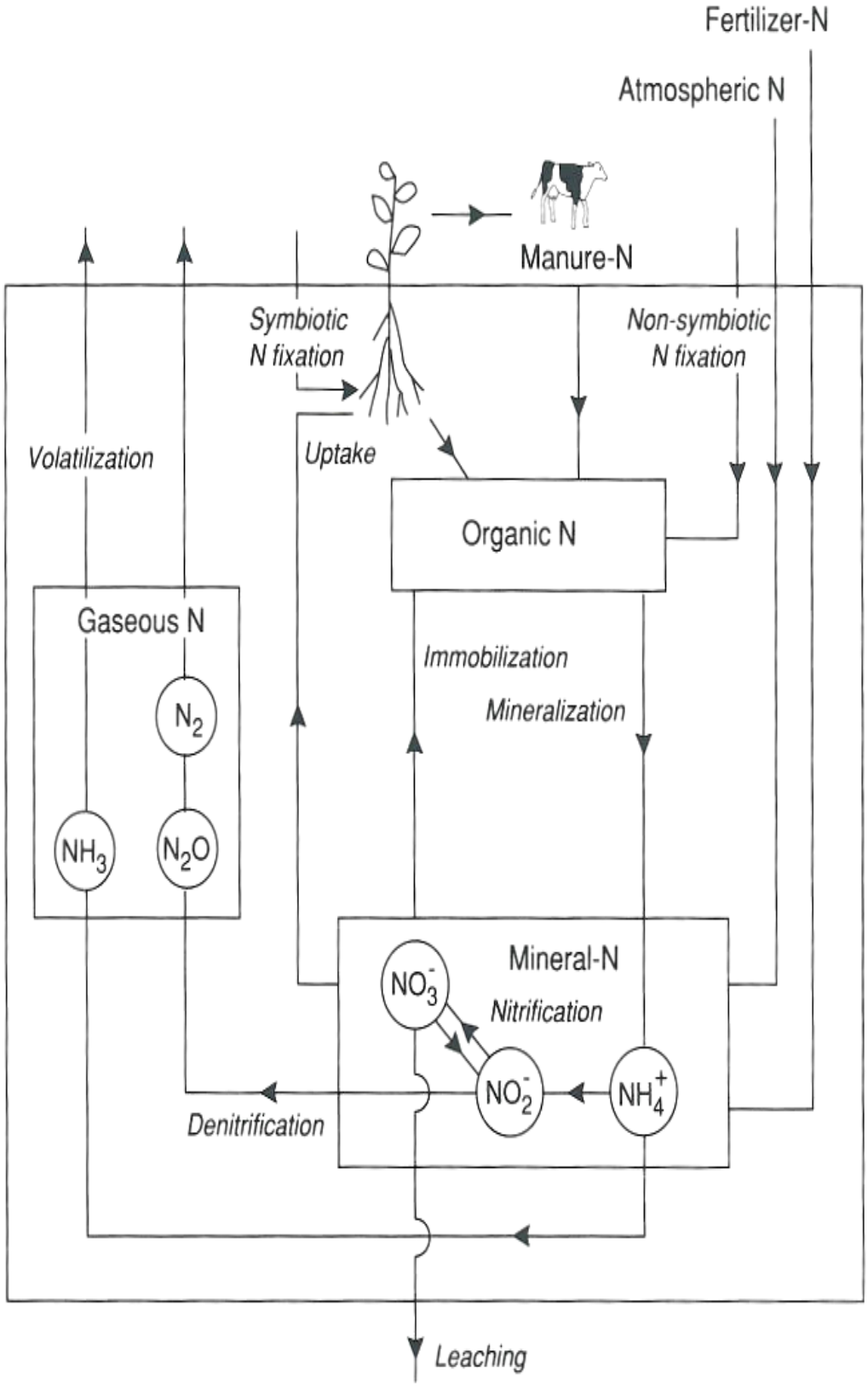

Figure 2.6. N cycle reproduced from Rowell (1994)

Table 2.2 N-Transformation processes involved during the mineralisation and immobilisation of N in soil

No.	N-Transformation processes	Type of reaction	Soil conditions	Optimum Temperature (ºC)	Optimum Moisture (% WHC)	Bacteria's Involved	N-Forms released	References
1	Ammonification	-		20	51	-	NH_4-N	(Aerts and Berendse, 1988, Sims, 2006, Moir, 2011)
2	Nitrification	Aerobic	Optimal pH is 7.5 - 8.5, presence of NH4-N and microorganisms and soil aeration	30 – 35	40	Nitrosospira, Nitrosococcus, and Nitrosovibrio involved in the first step. Nitrobacter and Nitrosospira -like bacteria are involved in the second step	NH_4-N to NO_2-N and then to NO_3-N	(Schmidt, 1982, Paul and Clark, 1996, Bartosch et al., 2002)
3	Denitrification	Anaerobic	Optimal pH is 6.0-6.5, waterlogged soil, presence of nitrate, decomposable organic matter and absent of oxygen	1.7 – 25	-	Pseudomonas and Clostridium	N_2O + NO +N_2	(Nelson, 1982, Tiedje, 1994, McKenney et al., 1997)
4	Volatilisation	-	pH < 7.5	45	-	-	NH_3-N	(Tisdale et al., 1985, McInnes, 1986, Schwab and Murdock., 2005)

2.7.1 Nitrogen mineralisation

Plants cannot directly use the organic form of N that is present in the soil. They can only use ammonium (NH_4-N) and nitrate NO_3-N) forms of N, typical inorganic forms of N (Pierzynski and Gehl, 2005). Prior to crop uptake, organic forms of N must be converted into afore-mentioned inorganic forms by a process called mineralisation or ammonification. Microbes present in the soil are constantly metabolising the organic matter and recycle the N during their process of breaking down organic matter. Organic N is typically present in the form of proteins, chitins, amino sugars and nucleic acids (Pierzynski and Gehl, 2005). Soil microorganisms, in general, hydrolyse these proteins or other organic N and convert them into ammonium ions ($NH4^+$), a process mediated specifically by heterotrophic soil microorganisms (Pierzynski and Gehl, 2005). The rate of mineralisation increases with an increase in temperature, moisture and with increasing soil pH (Chae and Tabatabai, 1986b). Therefore a wide range temperatures and moisture contents in soils have been studied and it was found that the rate of mineralisation is optimal in the temperatures range from 40 to 60 °C, while the optimal range for soil moisture content is between 50 and 75 % (Pierzynski and Gehl, 2005). Mineralisation can occur under anaerobic conditions also with the help of microorganisms.

2.7.2 Nitrification processes

Nitrification is the process, by which nitrate (NO_3-N) ions are formed by the oxidation of ammonium ions ($NH4^+$) by nitrifying bacteria under aerobic conditions. Therefore nitrification is an important process and it is important to understand the factors that favour nitrification. Nitrification is a two-step chemical reaction in the presence of oxygen and two types of nitrifying bacteria, nitrosomonas and nitrobacter. In the first step, ammonia-oxidising bacteria, such as Nitrosomonas spp. oxidise NH_3-N to nitrite ions (NO_2-N) (equation 3), which is further oxidised into nitrate ions (NO_3-N) by NO_2-N oxidising bacteria such as Nitrobacter spp., as given in equation 4 (Schwedt, 1996).

$$2NH_4^+ + 3O_2 + 2H_2O \longrightarrow 2NO_2^- + 4H_3O^+ \qquad \text{Eqn. 3}$$

$$2NO_2^- + O_2 \longrightarrow 2NO_3^- \qquad \text{Eqn. 4}$$

The pH of the soil is very important for this process because if the pH is greater than 9, the nitrite ion (NO_2-N) concentration may increase and nitrite can accumulate in the soil (Day et al., 1978). These ions are toxic to plants and may have an adverse effect on plant root growth. The optimal conditions reported for aerobic nitrification was between 30 and 35 °C, moisture content of 50 – 67 % and a pH between 6.6 and 8.0. Ammonium ions ($NH4^+$) can be either directly absorbed by plant roots before and after its oxidation into nitrate (NO_3-N) by nitrifying bacteria. Nitrifying bacteria are gram-negative 'chemoautotrophs' that are known to utilise carbon dioxide (CO_2) as a C source, which increases the rate of nitrification prior to N being absorbed into the root (Pierzynski and Gehl, 2005).

2.7.3 Nitrogen immobilisation

Microorganisms need N for the synthesis of compounds necessary for their growth, reproduction and survival. Inorganic forms of N (such as NO_3-N, NO_2-N and NH_4-N) are taken up by soil microorganisms and are transformed from the inorganic form into organic forms (Pierzynski and Gehl, 2005). When there is a high supply of available C from the organic matter in the soil, it will be stimulated microbial growth and which in turn because the microbial assimilation of available N. The C: N ratio of residuals organic content present in the soils is a key factor that controls N immobilisation within microbial biomass. In general, when organic residuals have a C:N ratio of > 20:1, that will result in net immobilisation of N into the microbial biomass and when the ratio is < 20:1 there will be net release of N, which is available for crop uptake (Pierzynski and Gehl, 2005).

2.7.4 Nitrogen losses

The loss of N from the soil is denitrification, which is defined as the chemical reduction of mineral N forms such as of nitrate (NO_3^-N) or nitrite (NO_2^-N) into N_2 gas and lost into the atmosphere. This chemical reduction process is mediated by the chemoautotrophic bacteria present in the soil under anaerobic conditions. These bacteria use NO_3^- as an oxidant (as an alternative to oxygen) for organic matter decomposition under anaerobic conditions, and during this process these ions are reduced into gaseous N (Pierzynski and Gehl, 2005).

This denitrification process occurs readily when there are oxygen deficient conditions present in the soil. When there is high organic C availability, there will be a rapid

depletion of oxygen by soil microorganisms in waterlogged soil regions (Webb et al., 2004, Mendoza et al., 2006). The denitrification reaction occurs readily at approximately 30oC when the pH ranges between 7 – 8 (Sprent, 1987, Pierzynski and Gehl, 2005). Under highly acidic conditions especially when pH is lower than 3.5, nitrous oxide N2O is the major product as a result of denitrification (Sprent, 1987).

2.7.5 Leaching of nitrate (NO_3-N)

The amount of N leaching losses from the soils is mainly through the NO_3-N form because of the low inherent capacity of most soils to retain anions like nitrate. The mobility of ammonium ions ($NH4^+$) is lower than the mobility of NO_3-N in soils and are usually exchanged with the cations present in the clay soils, making them bound strongly to the negatively charged soil particles (Tester et al., 1977, Shen et al., 1997). NO_3-N leaching to groundwater contributes significantly to the N loss and contaminates groundwater (Addiscott, 2005). The parameters such as rainfall, N fertiliser application rate, irrigation, soil type, depth of the aquifer and geology needs to be considered as these parameters are known to affect the N leaching. Those agricultural lands which receive high rainfall, high applications of N fertiliser for intensive irrigation, have shallow soils with a high stone content and fissured rocks, such as limestone, and sandy soil types will be most susceptible to nitrate leaching (Webb et al., 1997, Pierzynski and Gehl, 2005). However, there are some of the soil types that can retain the NO_3-N and those soil types include silt soils with underlying rocks such as sandstone, chalk (Conry, 1997, Webb et al., 1997). Soils having a high organic matter content may have high water holding capacity (WHC) and hence the possibility of leaching (Addiscott, 2005). In a few cases, the perennial pasture (crop cover) decreases the NO_3-N reduces leaching losses while, ploughing grassland results in significant NO_3-N leaching (Addiscott, 2005). When the rate of precipitation exceeds soil infiltration, nitrate, ammonium ions rate, will be lost due to erosion of soil or by run-off of surface (Pierzynski and Gehl, 2005, Ojeda, 2006). Therefore, gradient of the land, soil structural properties, N application rate, and precipitation needs to be considered when erosion and runoff are the major forms of N leaching. Major contribution of ground water N contamination originates from the application of N containing fertilisers, organic wastes and manures.(Addiscott, 2005). Drinking of water that contaminated NO_3-N creates a disease called

methaemoglobinemia or ‘blue baby syndrome’ and this is very much prevalent in in children less than 1 year old. The nitrate ions are converted into NO_2-N by the microorganisms present in the stomach and this NO_2-N binds with haemoglobin strongly, which disables the metalloproteinase for O_2 transport around the body, to providing a form with impaired function, methaemoglobin. As a result, transport oxygen carrying capacity of the blood is significantly reduced and affected children face suffocation (Addiscott, 2005).Soil erosion and run off may enrich the surface and coastal waters with N that can lead to eutrophication and in particular, marine environments faces severe risk as a result of eutrophication. Due to eutrophication, there will be excess growth of weeds and algal blooms and poor quality water with depleted amount of oxygen, making it unsuitable for other aquatic life like-fish (Chambers et al., 2006).

2.7.6 Ammonia (NH_3-N) volatilisation

The N from in soil can also be lost in the form of ammonia gas. In some soil types due to the high soil pH, free ammonia concentration tends to increase, which eventually escape as gas (Pierzynski and Gehl, 2005). Other than the pH of the soil, dry weather, high temperature, low cation exchange capacity of the soil and wind speed are the other major factors that contribute to ammonia volatilisation (Freney et al., 1983, Pierzynski and Gehl, 2005). Moreover, the application of N containing manures, sludge and fertilisers onto the soil surface instead of incorporating within the soil also increases the rate of NH_3-N volatilisation (Beauchamp et al., 1978, Robinson and Polglase, 2000, Pierzynski and Gehl, 2005).

Loss of N in the form ammonia vapour creates another side effect in the formation of aerosols, wherein the vaporised ammonia reacts with sulphuric acid, to form soluble ammonium bisulphate $(NH_4)HSO_4$ and ammonium sulphate $(NH_4)_2SO_4$, which are solubilised during precipitation. Vaporised ammonia reacts with the hydroxyl radicals to form NO_x species; which promote the acidity of the rain water (Galbally and Roy, 1983). Deposition of ammonia in aquatic or terrestrial habitats due to its high solubility in water, can cause damage to aquatic life, wildlife and nutrient poor ecosystems (Roem et al., 2002).

2.7.7 Nitrogen input from atmosphere

Conversion of gaseous N_2 into chemically different forms through lightning strikes or the action of soil microorganisms is called N fixation and this is the major natural mechanism by which N is incorporated into soil. Rainfall is another visa of N transfer from atmosphere to soils. N fixing bacteria such as Rhizobium spp., microorganisms are known to live in the root nodules of most legumes and can convert the chemically inert N gas to ammonia (NH_3-N) by using the nitrogenase enzyme (Sprent, 1987). Another mechanism in which the atmospheric deposition of N may also occur to a small extent on dry particulate matter (Pierzynski and Gehl, 2005). In addition to these natural N fixation processes, application of minerals, organic fertilisers, animal excreta and plant residues on the soil are the other common methods of N inputs into the soil.

2.8 Nitrogen transformation involving the land application of biosolids

Biosolids N comes in both organic and inorganic forms. Inorganic N, mostly ammonium (NH_4-N) and nitrate (NO_3-N), is readily available to plants. Before organic N can be taken up, however, it must first be converted to inorganic forms. This process, which is completed by soil microbes as a by-product of organic matter decomposition, is called mineralisation. The mineralisation rate is therefore the rate at which organic N is made plant available. In biosolids, mineralisation accounts for much or most of crop needs. An understanding of the mineralisation rate concept can help improve biosolids management to meet crop N demands while minimizing the potential for regulatory concerns regarding groundwater pollution. The application rate of biosolids is calculated based on total N content in the biosolids

Nitrogen mineralisation estimated by Barry et al. (2006) has shown that 35 – 65 % of the total N applied at biosolids application rates of 1 – 4.5 NLBAR may be lost by gaseous emissions. However these losses were found to be decreased to 1 – 13 %, when application rates were reduced to 0.5 NLBAR. Application of certain kind biosolids into the soil under certain conditions may lead to rapid denitrification. For example, Mendoza et al. (2006) observed that rapid mineralisation of the biosolids and deep placement promoted rapid denitrification, when they used anaerobically digested cake of biosolids. There are few methods available to estimate quantitatively the gaseous N loses directly as a result of denitrification. To determine

the effects of polymer addition, soil and biosolids type, they conducted laboratory experiment to determine ammonia volatilisation and denitrification and estimated the total gaseous N losses (Pu et al., 2010). The major share of N loss observed from the denitrification process was 24 % and 29 % when anaerobic and aerobic biosolids were applied, respectively. On the other hand, N losses in the form of ammonia account less than 4 % to the total 35 – 65 % weight loss. Addition of polymer was found to increase the N loss to 29 % in the Vertosol and 7 % in the Ferrosol. Otherwise, heavy Vertosol (16 % total N applied) shows higher N loss than a Red Ferrosol (7 % of the total N applied). Most of the gaseous N contained > 90 % of the dinitrogen and the remaining < 10 % was likely to be nitrous oxide N_2O. Their main conclusion was that denitrification is the major form of gaseous N losses under warm and moist conditions.

Soil microorganisms play an essential function in the cycling of N within terrestrial ecosystems. Temporal variation in rates of N transformations results from seasonal changes in factors controlling microbial activity, like soil temperature, water potential, and C availability. Several studies suggest that seasonal variation in microbial biomass corresponds to variation in soil water potential and substrate (i.e., C) availability (Sims and Roswell, 1980, Singh et al., 1989, Srivastava, 1992). Few studies, have investigated the seasonal variation of microbial biomass within these ecosystems (Groffman et al., 1992). As a consequence, it is uncertain whether microbial biomass differs or remains constant during the growing season. Microbial activity within soil is thought to be most limited by C input from plant litter production (Smith and Paul, 1990). Under conditions of increased substrate availability, microbial populations could increase, provided that soil temperature and water potential do not limit growth. When biosolids applied to the soil, some of the inorganic forms of N are taken up by soil microorganisms and are transformed from the inorganic form into organic forms (Pierzynski and Gehl, 2005) which are will not be available to the crop. The ratio of C: N in the biosolids influences the rate of decomposition of organic matter and consequent immobilisation of soil N.

2.9 Factors affecting nitrogen dynamic processes in biosolids amended soil

The proportion of biosolids organic-N that is mineralised and the rate at which it becomes available is dependent on several factors involving environmental conditions such as soil moisture (Sierra et al., 2001a, Rahman and Rashid, 2002, Wennman and Kätterer, 2006), soil temperature (Smith et al., 1998a, Smith et al., 1998b, Smith et al., 1998c, Wennman and Kätterer, 2006) and pH (Tester et al., 1977) and soil type (Tester et al., 1977, Smith et al., 1998a, HernÃ¡ndez et al., 2002, Breedon et al., 2003) as well as the treatment of biosolids (Smith et al., 1998a, Morris et al., 2003, Pu et al., 2008, Rigby et al., 2010).

The total available N under field conditions can be used to estimate the plant available N in soils treated with biosolids. Since these measurements are influenced by different environmental conditions, it is important to repeat these studies for more than two years to account for seasonal variation (Bitzer and Sims, 1988, Jackson and Smith, 1997, Beckwith et al., 1998, Cogger et al., 1999, Smith and Durham, 2002, Morris et al., 2003, Gilmour et al., 2003, O'Connor et al., 2004, Cogger et al., 2004, Pritchard, 2005a, Rigby et al., 2009). A more realistic assessment of the residual nutrient availability in the years after the soil amendment can be made from field trials (Soon et al., 1978, Lindemann and Cardenas, 1984, Boyle and Paul, 1989, Smith and Durham, 2002, Morris et al., 2003, Pansu et al., 2003, Pu et al., 2008, Eldridge et al., 2008).

2.9.1 The effect of the biosolids and soil types on nitrogen dynamic in receiving soils

The results of a survey of reported laboratory incubation studies carried out on a variety of biosolids are presented in Figure 2.7 (References and notes in Appendix A, Table A 2). The figure shows the mean and range of mineralisable N of the total N and the mean N % values. In general, laboratory incubation studies are carried out from 42 to 480 days. Within the first 50 days of laboratory incubation studies, a significant portion of organic N appears to be released, especially if higher temperatures (> 25 °C) are used (Smith et al., 1998a, Smith et al., 1998c, Smith et al., 1998b, Smith and Durham, 2002). Since there is the presence of very slowly mineralisable fractions of organic N, a longer time period is required for the full

characterisation. Despite the fact that application of raw un-stabilised sludge is not permitted in most developed countries including Australia (US EPA, 1993, NSW EPA, 2000, DEP and DOH, 2002, EPA Q and PWS Q, 2002, EPA Victoria, 2004, SA EPA, 2009), the data for such un-stabilised sludge is shown in Figure 2.7 as a comparison.

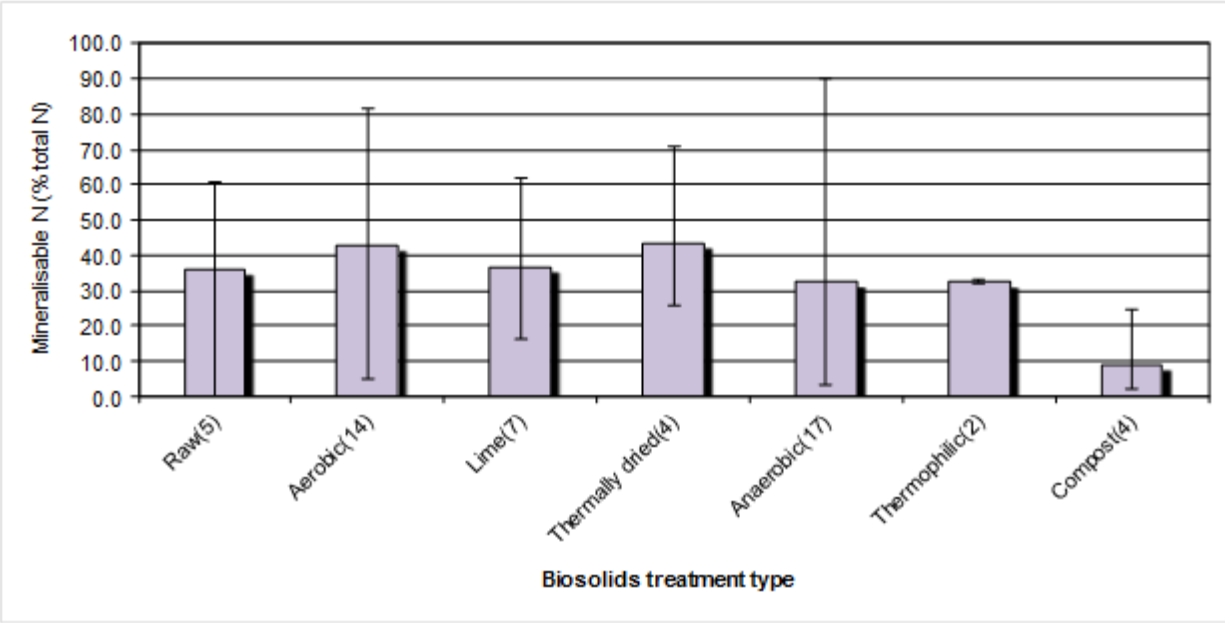

Figure 2.7. Mean mineralisable N (% total N) in various biosolids types determined through laboratory incubation studies (Appendix A, Table A 2). Number of samples measured for total N in parenthesis (within each sample there may have been laboratory replication); bars show the range of mineralisable N values reported.

Source: (Land application biosolids, Nitrogen and phosphorus management. final report as per Milestone 2, RMIT with the support of Smart Water Fund, Project 612-001)

During the digestion process of wastewater, a major fraction of the reactive organic matter is completely decomposed, to ammonia; this is the major reason why mesophilic anaerobically digested sludge has smaller mineralisable N content than mineralised content of un-stabilised sludge, with an overall mean value of 33 % (3.2 – 90 %). It is noteworthy that the variation among different sludge types is so wide, it makes these estimations meaningless. The average of the N mineralisation rate in anaerobically digested biosolids following the application rate in the first year compared to the guideline of biosolids land application rate in Australia are presented in Figure 2.8. This average mineralisable N content for mesophilic

anaerobically digested biosolids is approximately double the guidelines provided by the NSW EPA (NSW EPA, 2000), shown by the green line in Figure 2.8.

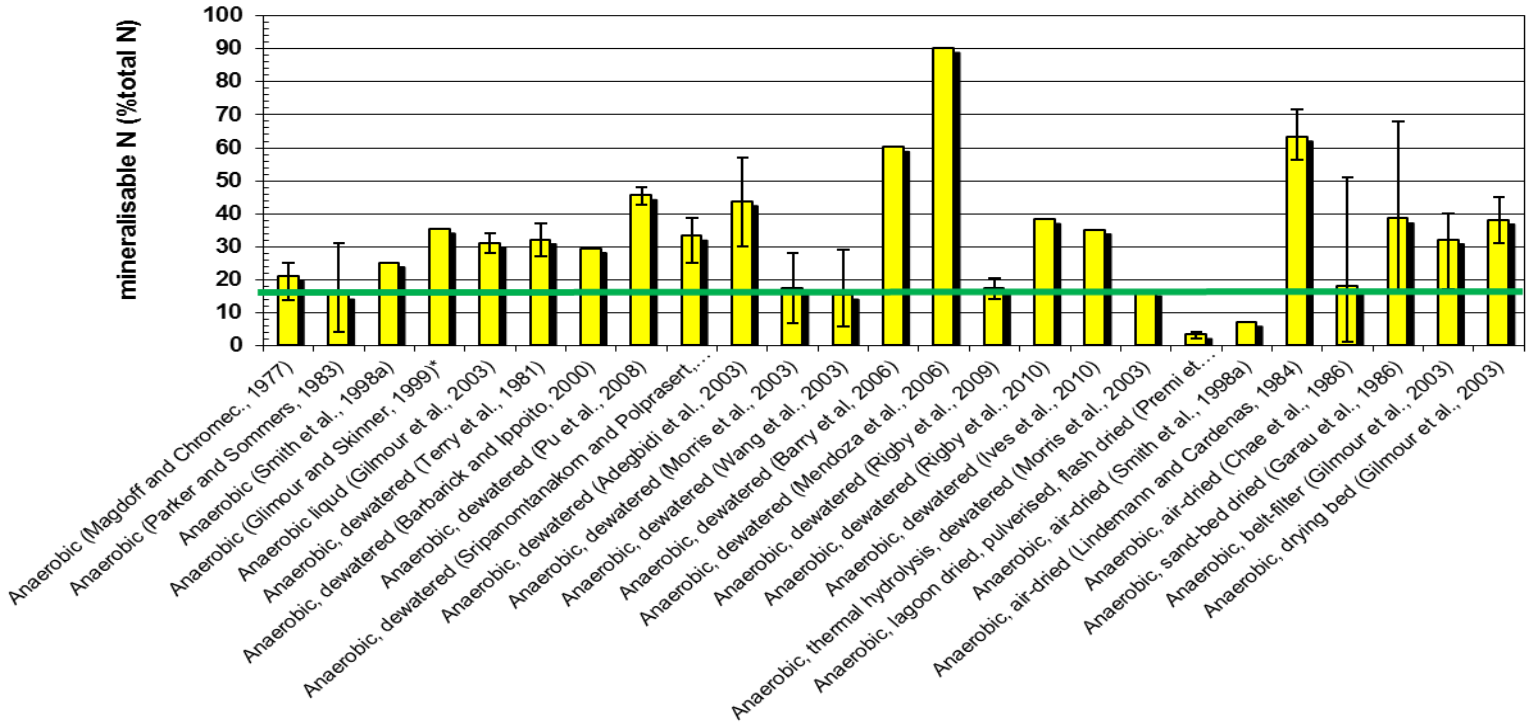

Figure 2.8. Mean and range of mineralisable N (% total N) content of anaerobic biosolids (Appendix A, Table A 2), compared to recommended first year mineralisable N in the Australian biosolids guidelines (thick green line); field investigations represented by green line

†Mineralisable N was calculated from nitrogen equivalency value and mineral N/organic content of biosolids (assuming 50 % volatilisation of ammonia). *Source: (Land application biosolids, Nitrogen and phosphorus management. final report as per Milestone 2, RMIT with the support of Smart Water Fund, Project 612-001)*

Biosolids obtained from both thermophilic anaerobic/aerobically digestion methods have comparable mineralisable N content (range 32.1 – 33.0 %) to the biosolids obtained from mesophilic anaerobic digestion (32.6 %). However, the mineralisable N content present in biosolids obtained from aerobic digestion have 43.1% (range 5 – 81.7 %), much higher than the recommended value of 25 % , as given in the biosolids guidelines (NSW EPA, 2000). The N mineralisation rate in aerobically digested biosolids are shown in Figure 2.9 and compared to the values reported in the literature. The greater mineralisable N content found in aerobically digested biosolids and un-stabilised sludge, clearly shows that the treatment process is not as effective at stabilisation of the organic matter content as anaerobic digestion. The average mineralisable N content of aerobically digested sludge is greater than that of the mineralisable N content of un-stabilised sludge (36.2 %). This increase might be possible that when un-stabilised sludge is added to soil, there is a resultant immobilisation to microbial N which will reduce the mineralisation. On the other hand, it seems that aerobic digestion modulates the chemical structure of few recalcitrant organic N forms in sludge increasing the size of the readily mineralisable N pool (Sierra et al., 2001a).

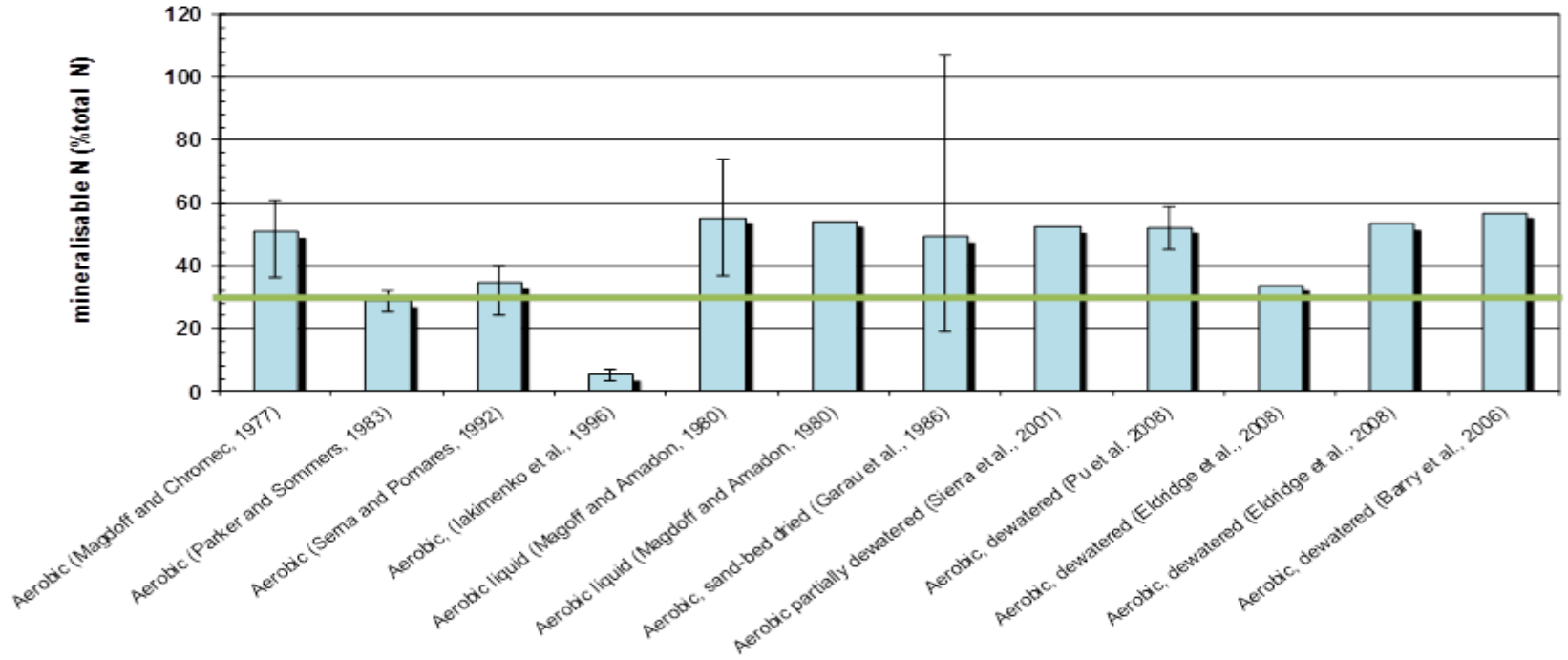

Figure 2.9. Mean and range of mineralisable N (% total N) content of aerobic biosolids (Appendix A, Table A 2), compared to recommended first year mineralisable N in the Australian biosolids guidelines (thick green line)

Source: (Land application biosolids, Nitrogen and phosphorus management. final report as per Milestone 2, RMIT with the support of Smart Water Fund, Project 612-001)

The values reported in the literature are highly variable, even within specific types of biosolids. Dewatering the biosolids and duration of storage are the main reasons proposed by Gilmour *et al.,* (2003) for the high degree of variability observed in mineralisable N value. In the case of air dried or lagoon dried biosolids, the mineralisable N values were found to be low due to a long drying period with loss of ammonia as a result of further mineralisation of organic N.

In the case of liquid digested sewage sludge after a year of storage, the mineralisable N content dropped from 15 % to 9 % after a year of storage (Coker et al., 1987). Figure 2.8 shows that anaerobic lagoon dried, pulverised and flash dried biosolids (Premi and Cornfield, 1971), anaerobic air dried (Smith et al., 1998a), and anaerobic air dried show the lowest mineralisable N values (Chae and Tabatabai, 1986b). Smith *et al.,* (1998a) estimated that 7 – 25 % of organic N was mineralised after incubation of 73 days at 25 °C in the case of anaerobically digested air-dried biosolids and mechanically dewatered, anaerobically digested biosolids respectively.

Despite the fact that dewatering affects the mineralisable N content, the method of dewatering was not described in many of the reports. The mean mineralisable N content of the biosolids that were obtained after lime treatment biosolids was found to be 36.8 % (range 16.3 – 62.0 %) a value higher than the mineralisable N content of anaerobically digested biosolids and close to value of aerobic and un-stabilised biosolids.

Addition of lime directly to the un-stabilised sludge, followed by the aerobic digestion, usually results in a lower level of stabilisation of the organic matter than sludge, which are first subjected to digestion (Figure 2.9). This was supported from the work of Mendoza *et al.* (2006) , wherein lime treated aerobic biosolids had a much higher mineralisation (62 %) of organic N than anaerobically digested lime treated biosolids (8 – 39 %) (Parker and Sommers, 1983, Gilmour et al., 2003).

Thermally dried biosolids had higher mineralisable N content of 43.7%, similar to the un-stabilised, aerobic and lime treated biosolids (range 12.1 – 71.0 %). *Sierra et al.,* (2001b) (Figure 2.10) have reported 71 % of organic N mineralisation , highest mineralisable N content during the period over 168 days in non-leached soil incubation. This high value might be a result of heat drying the sludge at 60 °C than other higher drying temperatures (> 100 °C) that are usually used for effective

sterilisation (Smith, 1996). Gilmour *et al.,* (2003) reported the lower mineralisable N content of 26 % over 75 days in a non-leached laboratory incubation procedure (Gilmour et al., 2003). Thermally dried biosolids even though they have lower mineral N content due to losses of ammoniacal N, have a larger pool of mineralisable N than anaerobically digested biosolids, which makes them a suitable source of N. Smith and Durham (2002) have shown that 30 – 60 % of the total N content was available in thermally dried biosolids, than dewatered digested biosolids that have only 30 – 40 % available. They indicated that the observation of larger and readily mineralisable pools of N in comparison to conventional biosolids may be due to the thermal drying causing fundamental changes to the organic matter present in biosolids. Eldridge *et al.,* (2008) reported the rate constant for the first order reaction 0.31 d^{-1} (range 0.25 – 0.43) that demonstrates the rapidly mineralisable fraction of organic N in thermally dried biosolids. Composted biosolids were found to have the lowest mineralisable N content of 9.3 % (2 – 24.5 %) and the value is similar to the value of 10 % of the recommended first year mineralisation as given by the Australian State Biosolids Guidelines.

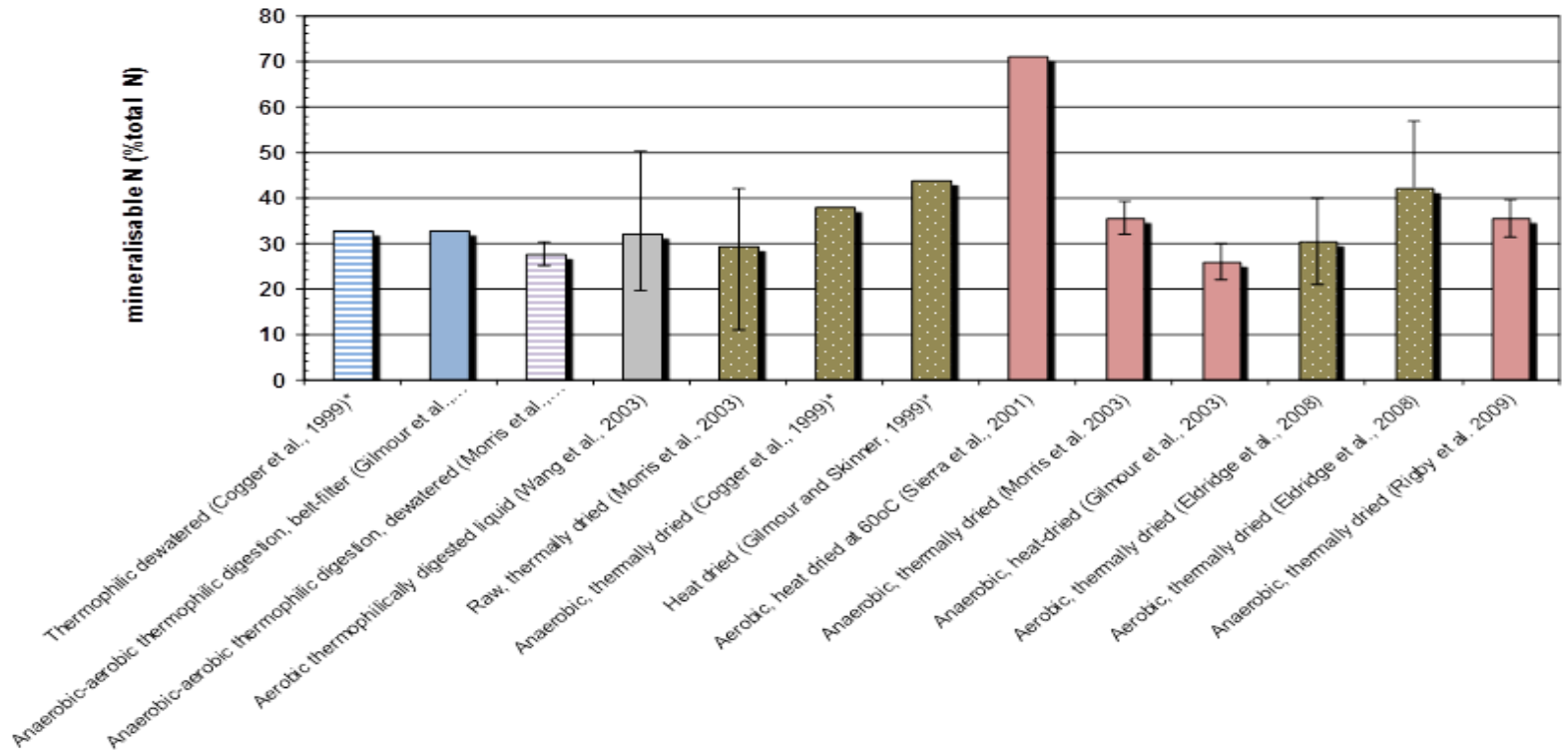

Figure 2.10. Mean and range of mineralisable N (% total N) content of thermophilic anaerobically digested biosolids and thermally dried biosolids (Appendix A, Table A 2).

*Mineralisable N was calculated from nitrogen equivalency value and mineral N/organic content of biosolids (assuming 50% volatilisation of ammonia)

Source: (Land application biosolids, Nitrogen and phosphorus management. final report as per Milestone 2, RMIT with the support of Smart Water Fund, Project 612-001)

The C to N ratio is a good predictor of Plant Available Nitrogen (PAN) in biosolids. Gilmour and Skinner (1999) estimated the PAN in 6 different biosolids with C:N ratios between 4 – 9.6 and the PAN was linearly related to biosolids C: N ratio, organic-N and total N. In a study by Parker and Sommers (1983), they showed that the amount of mineralisable N in sludge/biosolids was proportional to the total organic N present in the biosolids. In the case of composting and anaerobic digestion methods, which have a lower organic N concentration that undergoes further degradation after addition to soil? Overall, N mineralisation rate spans wide range of values for different kinds of biosolids and it was shown that these variations are due to the factors such as rate of application, soil type, temperature, moisture and pH. The relationship between biosolids application rate, soil type, temperature, moisture and pH factors and the observed variability in the mineralisable N needs further investigation. The variability mainly originates from the biosolids treatment processes within each biosolids types and several authors have investigated these factors in detail, results are given in the following section.

Soil type is the major factor that can influence the rate and extent of N mineralisation of biosolids (Chae and Tabatabai, 1986b, Wang et al., 2003a). Tester et al. (1977) reported that net N mineralisation (0 – 6 %) from organic N present in the case of biosolids-compost was higher in the case of loamy sand than a silty clay loam or a silty loam.

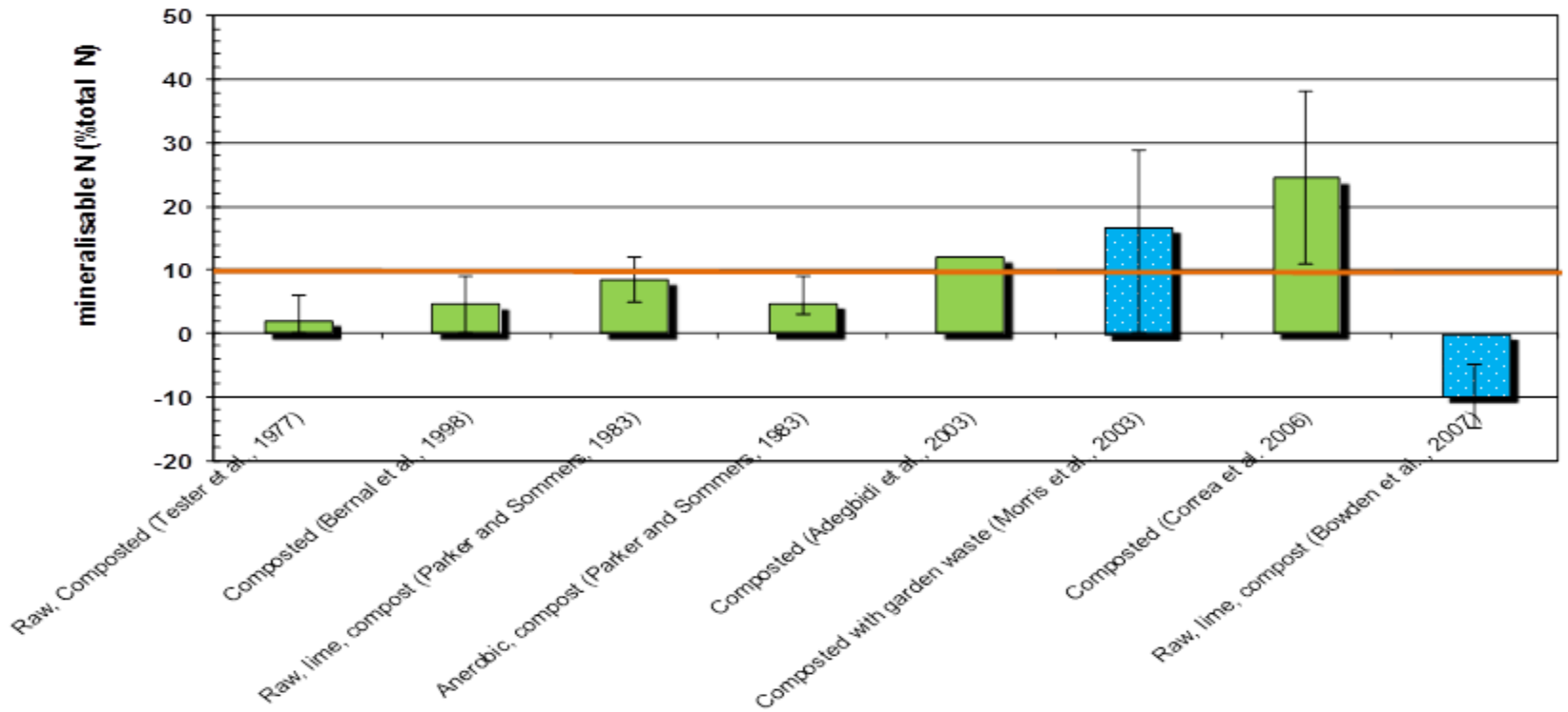

Figure 2.11. Mean and range of mineralisable N (% total N) content of composted biosolids (Appendix A, Table A 2); field investigations represented by orang line

Source: (Land application biosolids, Nitrogen and phosphorus management. final report as per Milestone 2, RMIT with the support of Smart Water Fund, Project 612-001)

In the case of silty loam, vermiculte clay fractions are responsible for NH_4-N fixation, but in the silty clay loam the authors suggested the lower nitrate recovery was due to the microbial immobilisation of N because this type of kaolinitic clay is not normally associated with $NH4^+$ fixation. Lighter textured sandy soil types in general enhance higher N mineralisation rates than the solids containing higher clay content (Hall, 1983a, Hernández et al., 2002) possibly because of better aeration in lighter textured soils. Aeration alone is not the point clay soils can also be highly aerated. Clay protects organic particles from microbial attack. On the other hand, mineralisation and nitrification rates may be greater in soils with higher clay content, due to the presence of highly active microbial population containing organic matter content (Chae and Tabatabai, 1986b, Jha et al., 1996a, Silva et al., 2005a, Correa et al., 2006, Rigby et al., 2009). Rigby *et al.,* (2009) carried out field investigations to understand the role of soil type on N mineralisation using a range of biosolids types. The study concluded that in the case of silty clay soil there was a greater initial mineralisation and nitrification rate with larger organic matter content than a sandy silt loam having only half organic matter content. In contrast, the final recoveries of mineral N in biosolids amended soil were greater in silty clay soil than sandy silt loam soil (Figure 2.8, Figure 2.10 and Figure 2.12).

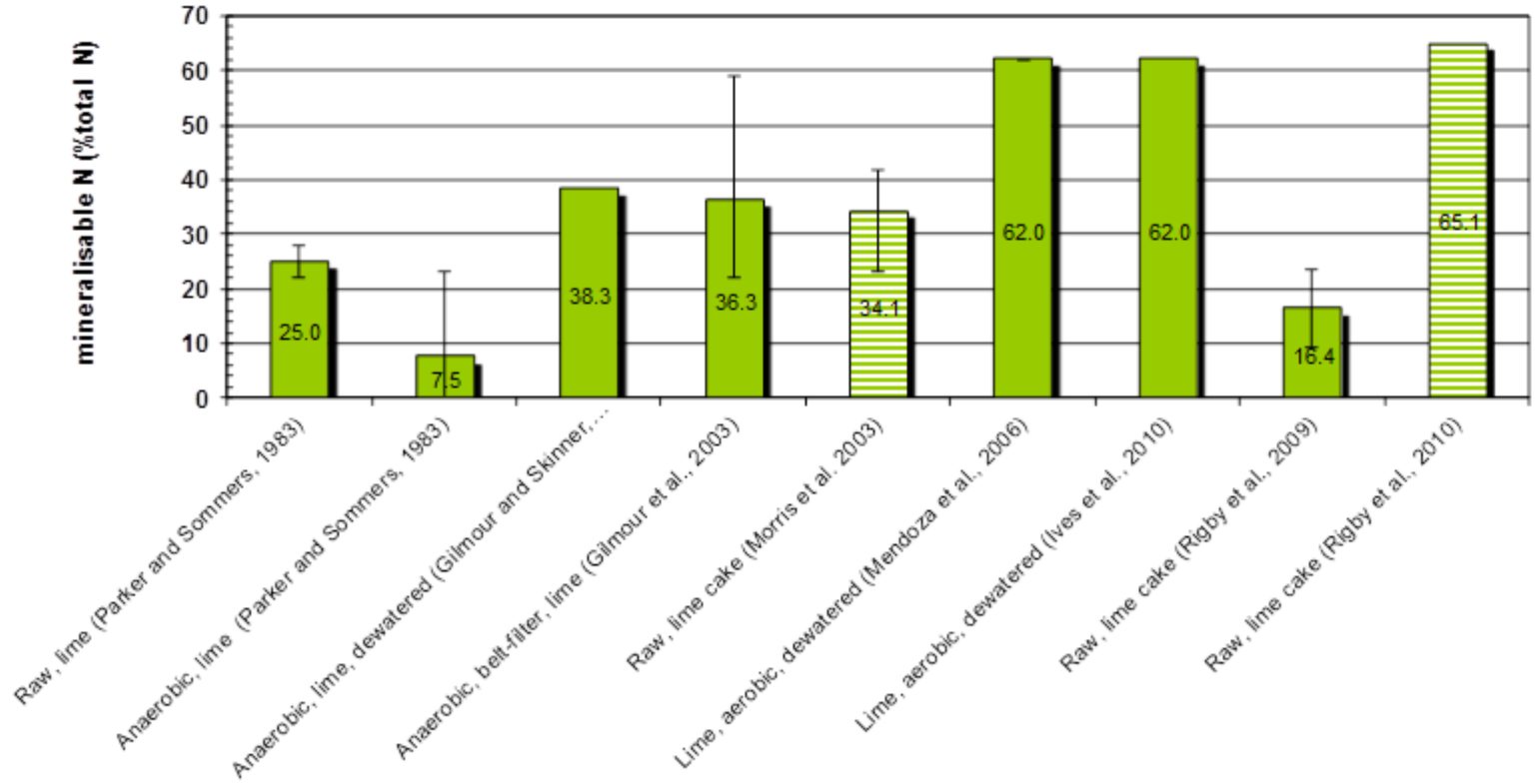

Figure 2.12. Mean and range of mineralisable N (% total N) content of lime treated biosolids (Appendix A, Table A 2);

Source: (Land application biosolids, Nitrogen and phosphorus management. final report as per Milestone 2, RMIT with the support of Smart Water Fund, Project 612-001)

2.9.2 Soil temperature

Soil temperature plays a key role in mineralisation as well as nitrification and an increase the soil temperature is known to increase the mineralisation of soil organic N and nitrification (Stanford et al., 1973, Wang et al., 2003a). In addition, temperature of the soil also influence the decomposition of biosolids and N transformations in biosolids amended soils (Gilmour and Gilmour, 1980, Terry et al., 1981, Honeycutt et al., 1991, Smith et al., 1998a, Smith et al., 1998b, Smith et al., 1998c, Sierra et al., 2001a). Sierra et al. (2001a) demonstrated that the rates of mineralisation and nitrification increased between 20 – 40 °C; however, the effect of temperature was greater on mineralisation and nitrification between 20 – 30 °C than 30 – 40 °C. In a laboratory incubation study, anaerobically digested biosolids were amended on three different soil types such as fine silt, silty clay loam and fine loam and the rate of mineralisation between the temperature of 15 – 30 °C was studied (Terry et al., 1981). Over 180 days of the incubation the amount of N mineralised at 30°C was three times greater than the N mineralisation at 15 °C. Similar results were obtained by Wang et al. (2003a) who reported that mineralisation of organic N in biosolids (Figure 2.8 and Figure 2.9) with forest soils over 26 weeks of a laboratory incubation period and it was found that rate of mineralisation was significantly greater at 20 °C (mean 22.8 %) than at 10 °C (mean 9.7 %). Ives et al. (2010) found that the fraction of organic N mineralised after 56 days was 35 % and 62 %for anaerobically digested and lime treated biosolids, respectively. However, it is well known that even at very low temperatures < 5 °C, N mineralisation can occur (Opperman et al., 1989, Cookson et al., 2002). Although slower rates of mineralisation are usually associated with very low temperatures, still there is a possibility of NO_3-N from biosolids amended solids in regions having cool and wet climatic conditions.

2.9.3 Soil moisture

The soil moisture level is another key parameter that has a strong effect on organic N mineralisation. At temperatures greater than 5 °C, an increase in the level of soil moisture enhances the rate of organic N mineralisation (Stanford and Epstein, 1974). However, the influence of moisture level on plant N availability from the biosolids amended soils is complicated. Pritchard and Rigby (2010) carried out short-

term laboratory incubation studies, wherein they observed that mineralisation occurs faster in a soil containing 100 % gravimetric water holding capacity than the soil having only 25 % of gravimetric water holding capacity. However, the rate of N mineralisation increased between 25 – 50 % due to the increase in moisture level, denitrification losses considerably reduced the net mineralised N content. In contrast, Hseih et al.(1981) did not observe any significant increase in rate of N mineralisation in a digested sewage sludge when they increased the moisture level from 6 – 100 % field capacity.

2.9.4 Soil pH

The pH of the soil also plays a pivotal role in N mineralisation rate and experimental studies have shown that the rate of N mineralisation increases with increasing the pH of the soil (Chae and Tabatabai, 1986b, Garau et al., 1986, Wang et al., 2003a, Hseu and Huang, 2005, Mendoza et al., 2006). Hseu and Huang *et al.,* (2005) chose three different types of soils of varying pH and amended them with anaerobic and aerobic biosolids and incubated the mixtures over 48 weeks. They found a higher rate of N mineralisation in the fine silty soil than in either the fine soil (pH 4.7) or the sandy soil (pH 5.7). In contrast, Terry *et al.,* (1981) did similar studies on a silt loam soil of varying pH values between 5.3 – 7.5, but they didn't observe any change in rate of N mineralization. Many soil oxidisers function between pH 7 to 9 (Tester et al., 1977), therefore the rate of nitrification is slower in the case of soils having a low pH. Due to the acidifying effect of nitrification, the land application of biosolids with a neutral or acidic pH may also reduce the pH of the soil (Terry et al., 1981, Wang et al., 2003a).

2.9.5 Effect of soil microbial populations

A high C: N ratio can inhibit the mineralisation and leads to immobilisation. Rates of mineralisation from N limited soil was found to be half of the rate of mineralisation from soil containing excess supply of N. Similar to the case of individual waste types, microbial immobilisation of nutrients in the soil was found to be increased when high concentrations of degradable C was present in the biosolids (Recous et al., 1990). Therefore, the biosolids with high C/N ratios may reduce N availability following application to soil. Bernal *et al.,* (1998) studied three sewage sludge-cotton waste composts at different stages of maturity, where they found out that the easily

decomposable organic C compounds in the raw waste led to N immobilisation, as the microbes attacking the fresh organic matter found a surplus of organic C with respect to organic N. This N immobilisation in soil caused a low yield of ryegrass plants due to N deficiency during the first weeks of growth (first harvest), as the concentration of N in plants did not reach the range 2.5 - 2.9 % for adequately fertilised ryegrass (Goh and Kee, 1978). Similar effects were observed by Calbrix *et al.,* (2007) , where they observed that partially stabilised biosolids that contains higher C content were a greater stimulus for soil microbial communities. It was established that there is definite relationship between the mineralisable organic matter, lignin content and microbial activity.

Soil type has a strong effect on the immobilisation of N and this was demonstrated in the study of Smith *et al.,* (1998b), when raw sludge was incubated with different soils. Soils with low organic content such as loamy sand had higher N immobilisation than the soils such as clay soils with high organic matter content. Breedon *et al.,* (2003) showed that the release of N was facilitated in highly fertile soils. They estimated that dewatered digested cakes released approximately 60 % and 45 % of total N in a highly fertile soil and in a less fertile soil, respectively, even though the exact mechanism was not elucidated. One possible explanation is due to the microbial populations present in fertile soils could adapted more readily than those present in less fertile soils than those present in less fertile soils. Another possible mechanism is that the microbial population present in the biomass tends to retain N in N starved systems. However, in the case of soils amended with biosolids (Petersen et al., 2003) were unable to establish any relationship between microbial biomass N (MBN), soil type and biosolids amendment. Nevertheless, these authors demonstrated that MBN was reduced when there is an accumulation of N in plant biomass. These results clearly indicated that soil N in biosolids amended soil contains significant fraction of MBN and an active source of N for plant uptake. Therefore, it is important to include MBN while investigating various soil effects on N availability from organic amendments.

2.10 General concept of the calculation biosolids land application rate

Environmental conditions and timing of the application rate might be restrict the application of N, which in turn puts a serious limitation on estimating the availability

of N content in biosolids. Parameters such as year of application, crop requirements, PAN and residual N present in the soil in the subsequent years need to be taken into account before biosolids are applied into the soil. In USA and UK, the regulations clearly state that the application rates of biosolids based on total N which it should provide the crop N requirements. In addition the timing of biosolids applications must be taken into consideration to reduce leaching and atmospheric losses, as stated by the USDA-NRCS Conservation Practice Standards (USDA, 1999, Pierzynski and Gehl, 2005). The afore-mentioned international regulations on biosolids application rate based on the N content are also followed in the Australian guidelines. The Nitrogen Loading Application Rate (NLAR) (DPIWE, 1999, EPA Victoria, 2004, SA EPA, 2009) or Nitrogen Limited Biosolids Application Rate (NLBAR) (NSW EPA, 2000, DEP, 2002) is expressed in dry tonne per hectare. Therefore, the amount of PAN applied in the biosolids must match the crop N requirement in the year of application (Equation 6).

$$NLBAR\ (t\ ha^{-1}) = CNR\ (kg\ ha^{-1}) / PAN\ (kg\ t^{-1}) \quad \text{Eqn. 6}$$

Where:

NLBAR= Nitrogen Limited Biosolids Application Rate ($t\ ha^{-1}$)

CNR= Crop N requirement ($kg\ ha^{-1}$)

PAN= Plant available N ($kg\ t^{-1}$) calculated according to Equation 7.

$$PAN\ (Year\ 1) = NH_4\text{-}N + (NO_3\text{-}N + NO_2\text{-}N) + (Min\text{-}N \times Organic\ N) \quad \text{Eqn. 7}$$

Where:

Min-N= fraction organic-N mineralised

In the first year of application, total mineralisable N fraction of the organic N pool ranges between 10 – 25 % according to the NSW EPA (2000), depending upon type of biosolids; this is similar to the values recommended by the US EPA guidelines (US EPA, 1997) (Table 2.3). However, the mineralisable N fractions obtained from US guidelines (Darvodelsky, 2009), are not adapted to the Australian climatic conditions. Even though there are no recommended values for the second year mineralisation rate, it is important to calculate the residual N in places where the application of biosolids is frequent. (NSW EPA, 2000).

Table 2.3 Estimated organic N mineralisation in the first year of application, based on biosolids type (NSW EPA, 2000, US EPA, 1997)

Biosolids type	Mineralisable N (Australian Guidelines)	Mineralisable N (US Guidelines)
Aerobically digested	25 %	30 %
Anaerobically digested	15 %	20 %
Composted	10 %	10 %

Except for Western Australia, all other Australian States have adopted these values. In Western Australia, at least 15 % of the organic N mineralisation is presumed regardless of the type of biosolids used for land application (DEP and DOH, 2002).

Due to the lower ambient temperature in Tasmania, 15% organic N mineralisation is suggested (DPIWE, 1999) probably due to the lower N mineralisation values. The New Zealand national guidelines (NZWWA, 2003) restrict biosolids applications on pasture to an annual limit of 200 kg total N ha^{-1} as an average over a period of three years. These regulations significantly reduce the risk of N losses through leaching and runoff. The US guidelines take into account the loss of ammonia through volatilisation as a factor in the calculation of the PAN value, which ranges from 0 – 50 % depending on the biosolids type and application method (US EPA, 1997). In the case of surface applied liquid or dewatered sludge, NH_3-N volatilisation has been measured as a loss of 50 % of NH_4-N (US EPA, 1997). However, when liquid sludge is injected into the soil, 100% of ammonium content was found to be fully available (US EPA, 1997). Australian Biosolids Guidelines does not consider ammonia volatilisation in the NLBAR calculation. However, in practice a volatilisation loss of 20 % (Pritchard, 2005a) and 50 % (Penney, 1999, Pritchard, 2005a) has been factored into the NLBAR calculations.

2.11 Techniques of quantifying or measuring the nitrogen dynamics in biosolids-treated soil

The availability of organic N from biosolids, or other organic wastes, with or without a crop, can be estimated by calculation of an N mass balance (Mamo et al., 1999,Torstensson and Aronsson, 2000, Pu et al., 2008). An alternative method, used in this study, is to estimate the PAN content of biosolids under field or glasshouse conditions by comparing the crop response (in terms of yield or N uptake) with the

crop response to a N fertiliser (Barbarick and Ippolito, 2000, Smith and Durham, 2002, Morris et al., 2003, O'Connor et al., 2004, Pritchard, 2005a, Barbarick and Ippolito, 2007, Rigby et al., 2010).

2.11.1 Laboratory incubation of biosolids amended soil

Laboratory soil incubation studies are a widely used technique to estimate the percentage of mineralisable N and the effects of variables such as application rate, soil properties, temperature and moisture under controlled conditions (Magdoff and Chromec, 1977, Magdoff and Amadon, 1980, Chae and Tabatabai, 1986b, Garau et al., 1986, Serna and Pomares, 1992, Smith et al., 1998a, Smith et al., 1998b, Smith et al., 1998c, Wang et al., 2003a). These incubation studies specifically aim to determine the extent of the total N mineralisable pool and the rate of mineralisation. These studies use either a leached or non-leached procedure to estimate the fraction of mineralisable N. In the case of the leached procedure, biosolids amended soil is first packed into a column and maintained in controlled conditions. This column is periodically flushed under vacuum with a dilute salt solution (typically 0.01 M $CaCl_2$) specifically to leach out the mineralised inorganic N. The leaching procedure has certain disadvantages, which were shown by Smith (1980) and Parker and Sommers (1983). In certain cases, even soluble organic N fraction (< 25 % of total N) was also be leached along with inorganic N, and may therefore underestimate the total mineralisable N. In contrast, Garau *et al.,* (1986) demonstrated that the leached procedure gave a higher estimate of mineralisable N than the non-leached procedure. This is possibly due to the continual removal of N from the system, potentially increasing mineralisation rate because incubation is close to the field conditions.

The alternative approach is a direct sampling of biosolids amended soil mixtures during incubation and the samples are subjected to extraction procedures to measure the different forms of mineral N. In another approach, in situ N mineralisation can be determined by sampling the soil in the absence of a crop (Eldridge et al., 2008, Rigby et al., 2009). This method uses PVC tubing, which isolates the treatments from plant root access and it has an advantage that these studies can be extended to a field trial.

First order kinetic models are regularly used to explain the change in mineralised N (Nm) in soils with respect to time (t) compared to the rate of N mineralisation (k) and the amount of potentially mineralisable organic N (No), (Stanford and Smith, 1972, Smith et al., 1980, Garau et al., 1986, Bernal et al., 1998) (equation 8):

$dNm/dt = k(N_o)$ Eqn. 8

NO_3-N and NH_4-N mineralised from the organic N fraction in soils over time, extracted or leached can be used to estimate the potentially mineralisable N fraction (No) in laboratory incubation studies. Rate of N mineralisation (k) mainly depends upon the soil type and environmental conditions such as moisture, temperature and pH (Pierzynski and Gehl, 2005). If these two values (No and k) are known for a particular soil type and set of environmental conditions, the amount of mineralised N in soil after a given time (t) can be estimated using equation 9.

$m = No\ [1-\exp(-kt)]$ Eqn. 9

2.12 Nitrogen transformation in the land receiving biosolids under field conditions

2.12.1 Nitrogen Mass balance

To estimate the availability of N in soil treated with biosolids, or other organic wastes, either in the presence or absence of a crop, a N budget or material balance needs to be calculated (Mamo et al., 1999, Torstensson and Aronsson, 2000, Pu et al., 2008, Pu et al., 2012). The N mass balance method estimate the proportion of mineralisable N (Barry, 2006) (equation 10). The N budget includes the proportion of N in biosolids present in different fractions in the soil such as organic N and mineral N (NO_3-N and NH_4-N), the amount of N fixed by the microbial biomass (MBN), the amount of N absorbed by the crop (Ncrop) and the amount of N lost through ammonia volatilisation (Nv) and denitrification (Nd). This method should provide realistic assessment of the rate and extent of N mineralisation under glasshouse or preferably field conditions. Under practical conditions, it is hard to obtain N quantities from different fractions and amount of N attributed to the unaccounted N gaseous emissions. Moreover, it is difficult to measure the residual organic N especially when the soil has high organic content (<0.5%).

Nm=100*([(Ncrop+MBN+minN+Nd+Nv) - (biosolids min-N)]/ (biosolids org-N)) Eqn. 10 (Barry, 2006)

Where: Nm=mineralised N

Ncrop = crop uptake of N

minN = soil mineral N of biosolids origin remaining in the soil

MBN= microbial biomass N of biosolids origin

Nd= N lost by denitrification

Nv= N lost by volatilisation

Biosolids are considered to provide a source of nutrients and mineralisation of organic nutrients slowly in the second and subsequent years after their application into the soil (Boyle and Paul, 1989, Binder et al., 2002). It is important to study the residual N content for one or more years in order to fully assess the total mineralisable N pool.

2.12.2 Nitrogen fertilizer equivalency

Another alternative technique to estimate PAN in biosolids is to compare the crop response (in terms of yield or N and P uptake) to the crop response to inorganic fertiliser N under field or greenhouse conditions (Barbarick and Ippolito, 2000, Smith and Durham, 2002, Morris et al., 2003, O'Connor et al., 2004, Pritchard, 2005a, Barbarick and Ippolito, 2007, Bowden et al., 2007, Rigby et al., 2010). This method determines the crop response to different rates of inorganic fertiliser N, ensuring that all other essential nutrients are provided according to the crop requirements except N. Basically these experiments are conducted in an N deficient soil, but supplying the other nutrients such as P, K, Mg, S and other trace elements. By using the regression equations, the N uptake with response to application rate of inorganic fertiliser N can be used to determine the PAN. A nutrient equivalency value or relative nutrient efficiency can be obtained by using the N uptake of the crops grown in biosolids-amended soil. In a second approach, this value can be obtained by producing a regression equation using several rates of biosolids application and comparing the regression coefficient to the coefficient from the inorganic fertiliser response curve to determine the N equivalency of the biosolids N (Barbarick and Ippolito, 2000). The N equivalency (Ne) of the biosolids is defined as an estimate of

the PAN fraction of the total N content of biosolids and can be used to estimate the proportion of organic N mineralised (Nm) as follows:

N_m (%) = (N_e - N_{min})/ N_{org} *100 Eqn. 11

Where: Nm (%) = proportion of biosolids organic N mineralised

N_e = nitrogen equivalency or PAN (%)

N_{min} = the proportion of inorganic N (NH_4-N+NO_3-N) (%) originally present in biosolids, this can be adjusted to include a factor for losses of N through NH3-N volatilisation (eg. 50%).

N_{org} = organic N (%) in biosolids

The estimated mineralisable N using this approach incorporates not only the mineralisable N that has been taken up by the crop but also includes the N lost from the soil profile by leaching, runoff, erosion and gaseous emissions.

2.13 Crop Nutrient requirements

Laboratory incubation studies of biosolids amended soils give insight into the key factors that affect the N mineralisation rate and extent of N mineralisation. However, in glasshouse or field conditions, the crop uptake of PAN in biosolids amended soil needs further investigation under specific site and specific environmental conditions. For example, the time of application of biosolids, method of incorporation and extent of incorporation may influence N availability. Another factor in field or glasshouse conditions is the residual N obtained by slow mineralisation of remaining biosolids The variations in temperature and rainfall during the application of biosolids may also greatly influence the reproducibility of crop response to biosolids (Morris et al., 2003).

2.13.1 International literature

There are many reports in the literature that have shown that biosolids could be an efficient source of N for crop growth (Cogger et al., 2001, Adjei and Rechcigl, 2002, Adegbidi and Briggs, 2003b, Morris et al., 2003, Akdeniz et al., 2006). The mean nitrogen equivalency of various biosolids types determined from the crop uptake and the results are presented in Figure 2.13 and these values are equivalent to the biosolids PAN content.

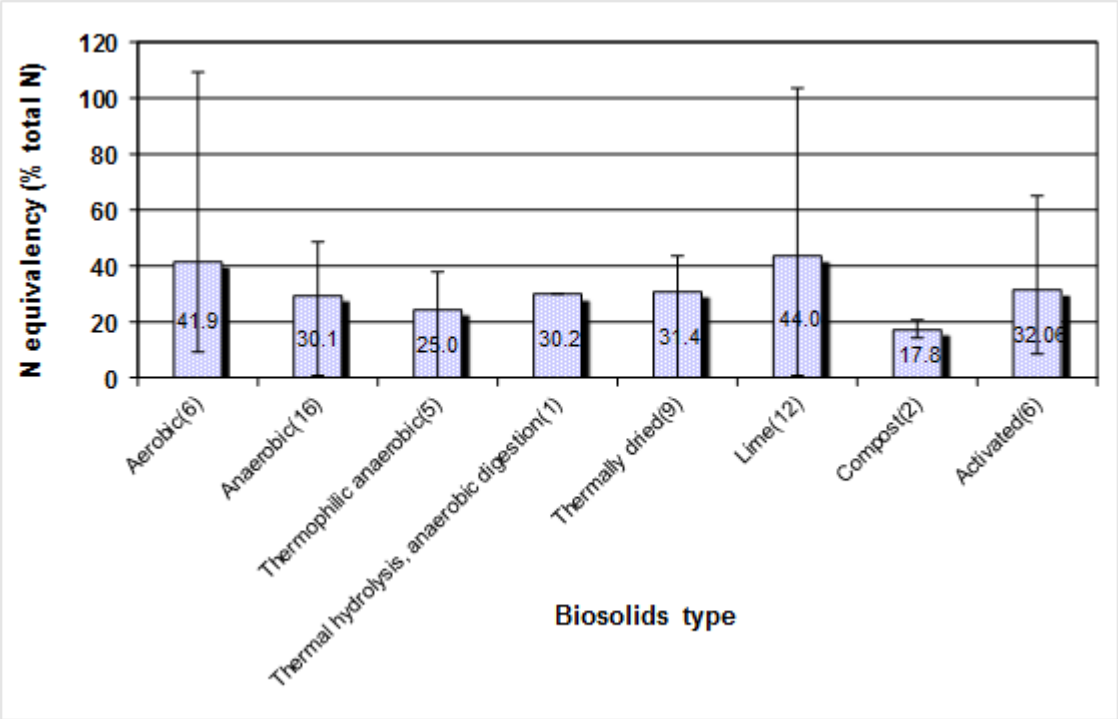

Figure 2.13. Mean nitrogen equivalency of various biosolids types determined through crop response trials, n in parenthesis

Source: (Land application biosolids, Nitrogen and phosphorus management. final report as per Milestone 2, RMIT with the support of Smart Water Fund, Project 612-001)

The crop uptake of N showed a similar pattern to the proportion of mineralisable N, wherein mineralisable fraction of organic N is the critical factor determining N availability (Figure 2.7). Lime treated biosolids and aerobically digested biosolids were shown to have the greatest amount of PAN (over 40 %) than the PAN of mesophilic anaerobically digested, thermophilic anaerobically digested, thermally dried. In the case of liquid biosolids, the N fertiliser value is dependent more on the NH_4-N content than the organic N fraction. Adjei and Rechcigl (2002) carried out an experiment on bahiagrass on a fine sandy soil to compare the agronomic value of lime treated liquid biosolids, lime treated biosolids caked, aerobically digested liquid biosolids and ammonium nitrate. These materials were supplied at rates of 90 and 180 kg N ha^{-1} and an unfertilised control, and they found that the forage production from the liquid biosolids was similar to that of the inorganic fertiliser, whereas yield in the solid biosolids treatments were approximately 30 % less.

The reproducibility of crop response to biosolids N applications was investigated by Morris *et al.,* (2003) who chose to work with perennial ryegrass as it has a long growing season and shows greater response to fertiliser N than most other crops. A wide range of biosolids were chosen with different treatments including conventional treatments, such as mesophilic anaerobic digestion and advanced treatments, such as thermal drying and thermal hydrolysis. In the case of ryegrass, biosolids and inorganic fertiliser were applied continuously for three years and the crop response for both ryegrass and inorganic fertiliser were compared. In these field trials, it was found that the ryegrass response against the biosolids for the period of the three years was relatively constant. Dewatered mesophilic anaerobically digested biosolids generally have a PAN content in the range 26.5 – 33.7 % of total organic N and this value is equivalent to 16 – 21 % organic mineralisable N. In the case of thermally dried mesophilic anaerobically digested biosolids, the range of PAN content was found to be 33.5 – 37.9 % of total N, equivalent to 31.9 – 36.7 % mineralisable N. In contrast, PAN from thermally dried raw biosolids was reduced from 35.5 – 42.3 % in the first and second years to 11.8 % in the third year, equivalent to 11.1 – 35.1 % mineralisable N. This may be attributed to the dry growing season in the third year, which resulted in reduced mineralisation of N. They have concluded that this effect is particularly noticeable in biosolids which weren't subjected to any biological stabilisation and therefore, contained large reserves of biodegradable organic matter. .

Barbarick and Ippolito (2000) conducted a 6 year investigation with wheat grown at two different sites, wherein they studied the N fertiliser equivalency from the anaerobically digested sand-bed dried biosolids. They found that R^2 from regression equations for the relationship between biosolids application rate and N grain uptake were generally highly significant, and in estimates of a period of 6 years there was greater variation in N uptake of wheat possibly due to drought and hail damage (Barbarick and Ippolito, 2007) with first-year N mineralisation rates in the range of 21 – 27 %. In a recent research carried out in US (Gilmour et al., 2003, Cogger et al., 2006) , it was indicated that the biosolids treatment process did not have any significant effect on PAN, unless they were subjected for an extensive stabilisation such as composting or lagoon storage. As many of the biosolids types are similar in nature Gilmour *et al.,* (2003) employed mean biosolids based on laboratory

decomposition and weather data to model PAN during the growth season across the US (Figure 2.14) and suggested that this is a more appropriate approach than defining PAN based on biosolids treatment process. US guidelines state that organic N mineralisation in dewatered mesophilic anaerobically digested biosolids was estimated to be 20, 10 and 5 % of biosolids in the first, second and third years, respectively. Similarly, Australian guidelines for the use of biosolids in agriculture state that second and third year mineralisation of biosolids N should be taken into consideration, but didn't mention about the N mineralisation (US EPA, 1997).

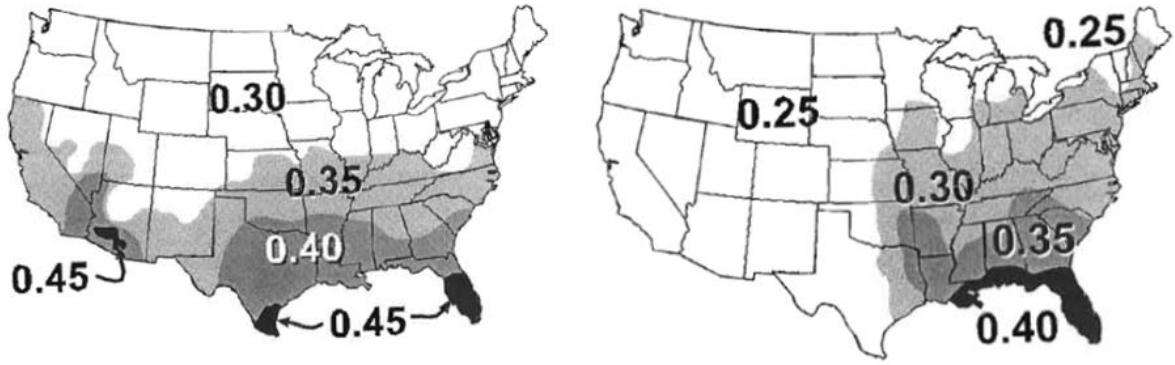

Figure 2.14. First year PAN (% total N) predicted for biosolids applied to irrigated (left) and non-irrigated cropland (right) reproduced from Cogger et al. (2006).

Observation of similar mineralisation rates both in laboratory incubations and field studies in the first year (Figure 2.7) suggests that the majority of the labile organic N pool may be available in the first year of application. However, Cogger *et al.,* (2006) have shown that the availability of N is controlled by the climate in the first year following application. Boyle and Paul (1989) found that there was still a mineralisable N pool remaining even after 3 years of application of an anaerobically digested cake. . Soon *et al.,* (1978) have shown that even after 3 years application of anaerobically digested sewage sludge (800 kg N ha^{-1}), they detected high concentrations of NO_3-N ion present in the soil samples that were taken at a 90 cm soil depth. Binder *et al.,* (2002) conducted a 4 year study to quantify the yield response to biosolids. They chose maize and rain-fed sorghum in eastern Nebraska. There were five rates of anaerobically digested biosolids compared to six rates of inorganic N at two sites on a silty clay loam soil. The increase in yield and the recovery efficiency of N in

response to biosolids was compared with inorganic fertiliser over the 4 years. . The N recoveries were shown to have similar trends in both maize and sorghum crops, wherein 33 %, 21 %, 14 % and 9 % increase in yield were observed for the first, second, third and fourth year respectively. The percentage of total N recovery by the crops in the first, second, third and fourth years was 40, 20, 10 and 5 %, respectively. The relative cumulative recovery of N was found to be dependent on the application rate. Much less N recovery (56% after three years) was observed in the case of an application rate of 50 t biosolids ha^{-1} while 75% recovery (after three years) was obtained when the application rate was 25 t ha^{-1}. Depending upon the climatic conditions, the relative fertiliser efficiency of biosolids in the second year may vary. Cogger *et al.,* (1999) carried out a field experiment to assess N recovery from heat-dried and dewatered biosolids applied to forage grasses and they found that substantial mineralisation in the second year. They observed that mean fertiliser N equivalency increased from 22 – 66 % at one site and 38 – 54 % at the second site. This is possibly due to dry conditions at the soil surface in the summer followed by cool winter that delayed the mineralisation until the following spring. . The first year PAN on a well-drained fine sandy loam at a site near Seattle estimated by Cogger *et al.,* (2004) for tall fescue growth was found to be similar (37 ± 5 %) for different kinds of biosolids (with the exception of lagooned biosolids). Depending upon the aging of biosolids, their PAN value also varied from 8 – 25 %. In some studies, it was shown that, PAN for the second year was dependent on the type of biosolids. For example dewatered biosolids had a PAN value of 13 ± 2 % for the second year while thermally dried biosolids had a PAN value of 8 ± 3 %. These results clearly demonstrate that most of organic N mineralised in the first year after application rate. Morris *et al.,* (2003) reported the very low residual N content in the second year field trials for most treated biosolids under temperate conditions and they estimated that less than 5 % mineralisation of total N for all different application rates occurred in the third year. Although the residual mineral N in the first year may be available for the second year, it was recommended to ignore the second year mineralisation values. This residual amount in the first year is usually taken into consideration when estimating the crop N requirements.

The method of application and timing of application should also be taken into account, in order to estimate the crop available N. In the UK, the N availability during

the spring season ranged between 50 % and 70 % as available as fertiliser N during the first year of application of sludge or slurries, whereas in early winter, its relative efficiency was considerably reduced to 20 – 30 % due to N leaching by winter rainfall (Hall, 1983a, Hall, 1983b, Hall, 1984). Compared to inorganic N fertilisers, biosolids release N slowly and are sometimes referred to as “slow source of source of N” (Adegbidi and Briggs, 2003a, Adegbidi et al., 2003). Adegbidi and Briggs (2003a) observed the release of N is increased in the case of biomass obtained from willow fertilised with lime treated biosolids than the commercial slow release N fertiliser. Biosolids that contain a smaller fraction of mineralisable N as well as slowly available N may limit crop growth due to the early season N deficiency. Bowden *et al.,* (2007) conducted a greenhouse study with tall fescue to determine N equivalency of composts and these values were compared to a N equivalency and mineralisation calibration curve of inorganic N fertiliser. It was observed that crop requirements and the N supply timing from the composts did not correspond very well. The extent of growth period of the selected crop also has a strong influence on N availability from the biosolids. Another study showed that cereals crop uptake of biosolids N required an amount, which was half of the biosolids N required for grass (Hall, 1983a). The water holding capacity and drainage characteristics of soil, in addition to the intrinsic soil-type, affect the crop available N.

2.13.2 Australian literature

In Australia, most research conducted on soils amended with biosolids has been undertaken by NSW agriculture, during a research program in the 1990s (Osborne et al., 1995) and during the National Biosolids Research Program (NBRP) established by CSIRO in 2002. Major studies on N availability were conducted in NSW (Eldridge et al., 2008, Eldridge et al., 2009), and detailed studies on P availability have been conducted in WA (Pritchard, 2005a).

Havilah *et al.,* (1996) carried out a field trial over the period of 3 years at the flood plain of the Shoalhaven River near Nowra, NSW and used urea and dewatered biosolids to see their relative effectiveness. N response studied on three major crops including sudan grass, maize and annual ryegrass and both urea and dewatered biosolids were applied at 6 different rates and. The mean uptake of N from urea was 59 – 66 %, while the uptake of N from biosolids was only 18 % by these crops.

These results proved that the relative efficiency of dewatered digested (24 – 28 %) is higher than urea. The NBRP initially focussed on heavy metals (Cd, Cu and Zn) and their environmental and crop risks in addition to the utilisation of organic and inorganic nutrients. Each of the five States involved employed the same experimental design at several different sites with one or more different biosolids types and crops, generally over 3 years. This design consisted of: an unfertilised control; a fertilised control with traditional commercial fertilisers applied annually at recommended rates; a series of biosolids rates (multiples of the NLBAR up to 4.5 NLBAR) applied once in year one prior to sowing the first crop; one rate of biosolids reapplied annually (1.5 NLBAR) to compare freshly applied biosolids with residual biosolids and metal salts treatments, not discussed here (Pritchard, 2005b, Whatmuff et al., 2005, Barry, 2006, Heemsbergen et al., 2007, Butler et al., 2007).

Victoria, as a part of the NBRP, obtained the biosolids from 9 wastewater treatment plants and was applied across 5 sites (Dookie, Dutson Downs, Melton, Mildura and Pakenham) as shown in Figure 2.15, using pasture and cereal crops.

Figure 2.15. Location of field experiments in the National Biosolids Research Program (Victoria State), (from McLaughlin et al., 2007a)

In the case of cereals, samples were collected for nutrient analysis either during two weeks prior to harvest or mid-tillering. However, for pasture, samples were collected during the growing season for more than 7 times. These samples were analysed for both nutrients and heavy metals content. Soil samples were collected from the surface (10 cm cores) for total N and P analysis and the soil sampling took place two weeks after application and two weeks before the harvest. In general, analysis of these samples give an insight about the biosolids' ability to supply the nutrients, crop response relative to inorganic fertilizer, despite the fact that it is difficult to obtain a quantitative estimation of N and P availability. Application of biosolids improved the production of dry matter significantly in the pasture trial, and they were found to be more effective than fertiliser in many cases. However, biosolids generally had a negative effect in the cropping trials and grain yield; although at a few sites there was a positive effect on dry matter and grain yield. The lack of moisture is

responsible for the negative effect on grain yields (Butler et al., 2007). In South Australia, air-dried and lagoon-dried anaerobically digested biosolids showed significant increases in NO_3-N concentration with increasing application rates at all sampling times. Overall, crops responded positively to biosolids, and the positive effects of biosolids application persisted for at least two years after the application of 1 NLBAR (Heemsbergen et al., 2007). Queensland among other Australian states, conducted detailed nutrient analysis under the NBRP. Five sites were chosen to study the application of aerobic and anaerobic biosolids continuously for the period of 3 – 4.5 years , and the production of grains, grain legumes, cotton, sugarcane and forages were part of these programs (Barry, 2006). In each trial site, partial nutrient budgets were calculated based on the fate of nutrients and metals. Biosolids application provided a significant increase in crop N accumulation, high residual N recovery at all sites, but a small proportion of N in harvested biomass, between 30 – 100 kg N ha^{-1}, equivalent to 2 – 11 % of total applied N. This may be the consequence of nutrient resources in the soil at the test sites. Moreover, these observations also provide an information at the end of the experimental period about the presence of small proportion of added organic N (from biosolids) in soil profiles (Barry, 2006).

The recoveries of N (harvested N, organic N and mineral N in the soil profile) after three successive crops were estimated from the N budget is less than 65 % of the total N applied and shows further decrease with increasing rate of biosolids (Barry, 2006). . At three of the four sites, 35 – 65 % of N gaseous losses from the total added N were the major contributing factor for N losses in aerobic and anaerobically digested dewatered biosolids, while there was no evidence of leaching losses. Additional experiments were conducted using maize as a test crop with lower application rates (equivalent to 0.5 NLBAR) and 87 - 99 % total applied N was recovered. This data showed that the mean mineralisation (over approximately 12 months) for both anaerobic and aerobic biosolids was 60 % and 57 %, respectively (Figure 2.8 & Figure 2.9). During the short term studies in a Red Ferrosol showed the N mineralisation rate was 58 %, for aerobic biosolids and 42 % obtained from anaerobic biosolids in the first 7 weeks after application. N recoveries showed significant increases in a continuous cropping system from 71 to 88 % N recoveries of the total N applied (Pu et al., 2008). The fate of N after biosolids application in a

cut and remove forage system on a heavy alluvial clay loam was examined over 8 months (Pu et al., 2008).

A N budget reveals that removal of crop accounted contributed 25 – 33 % applied N (1 NLBAR treatment), 38 – 51 % was residual N, 10 – 23 % was NO_3-N in the top 90 cm, and 12 – 29 % was lost through volatilisation or denitrification. The rate of organic N mineralisation was estimated to 52 % & 45 % for aerobic biosolids and anaerobic biosolids respectively (Figure 2.8 & Figure 2.9). Several laboratory studies were also carried out that involved the N mineralisation study on several dewatered anaerobically digested biosolids and thermally dried biosolids. These biosolids amended on two soils under anaerobic and aerobic conditions for 7 days, while the temperature was set at 40 °C and 30 °C for anaerobic and aerobic conditions respectively. For a period of 150 days in well watered soil in an aerobic glasshouse experiment organic N was 58 – 59 %. At shorter incubation periods, amount of mineralisation in an anaerobically digested biosolids was found to be less than aerobically digested biosolids and it was concluded that there were no obvious effects of biosolids treatment process (Barry, 2006).

The field studies that were undertaken in Queensland, have shown that rate of mineralisation in the first year is higher than the recommended 15 % and 25 % organic N for anaerobic and aerobically digested in the guidelines (EPA Q and PWS Q, 2002) and this may be the consequence of the warmer conditions of Queensland. Rates of mineralisation measured in Western Australia (Rigby et al., 2010, Pritchard et al., 2010) and NSW (Eldridge et al., 2008) were also found to be higher than the expected values. In South West Sydney, field trials were conducted on a silty clay loam soil, 45 – 54 % and 53 % of the proportion organic N mineralised for thermally dried biosolids and aerobically digested dewatered biosolids respectively. Importantly, most of the mineralisation completed rapidly at the start of the incubation (Eldridge et al., 2008). At the same site, the percentage of organic N mineralisation was measured from turf uptake of N over the period of 12 months and the results showed that thermally dried biosolids (Figure 2.12) and dewatered aerobic biosolids showed 21 – 45 % (Figure 2.9) and 34 % of the organic N was mineralisation respectively.

In Western Australia state, relative to urea as source of N, assessment of N release characteristics of three biosolids were studied using a field experiment (Rigby et al.,

2010) and a soil incubation study (Pritchard et al., 2010). The field experiment included six different application rates of urea and biosolids-amended acidic sand soil and ryegrass used as an indicator crop. The soil incubation studies were carried out in three moisture regions (25 %, 50 % and 100 % gravimetric water holding capacity (GWHC) and two kinds of soil.

The field trials indicated that the N availability of biosolids relative to urea was dependent on biosolids types, so the plant available N was greater in lime amended biosolids (65 %) (Figure 2.10) and alum sludge (63 %) than in the dewatered biosolids cake (39 %) (Figure 2.9).

Laboratory incubation studies also gave similar results, wherein lime amended biosolids (64 – 84 %), alum sludge (56 – 72 %) had a higher plant available N than the dewatered-biosolids cake (42-44%). The moisture content of the soil was found to be an influencing factor from the soil incubation studies and the rate of N mineralisation was found to be higher at 50 % GWHC than at 25 % GWHC, but in contrast at 100 % GWHC, losses of N were observed due to denitrification, especially from lime treated biosolids amended soil.

In field studies conducted in Tasmania (Ives et al., 2010), anaerobically digested biosolids and lime treated biosolids both showed N mineralisation rates of 35 % and 62 %, respectively The studies were designed to simulate spring and autumn conditions over 56 days, conducted at 12.5 °C and a soil moisture level of 70 % field capacity. The observations of Ives et al (2010) clearly showed that the rate of mineralisation suggested in the current guidelines may be an underestimate even for regions having cooler weather conditions.

To summarize, more laboratory and field trials need to be undertaken to study the Australian soil types and climatic conditions and their role in affecting the N bioavailability in biosolids-amended soils. During the NBRP, N uptake of crops was not estimated in the most of Australian States except Queensland (Pu et al., 2008) Subsequent, field trials have been conducted in Western Australia (Rigby et al., 2010) in New South Wales (Eldridge et al., 2008) to estimate the mineralisable N content the biosolids types, typical to those States. Most of the Australian studies showed the proportion of mineralisable N was generally found to be at the higher end of the range than field investigations conducted elsewhere in the world. Table

2.4 shows the mineralisation rate of organic N in the first year following the application of biosolids.

Table 2.4 Summarizers of findings using different technique to estimate the PAN in different biosolids treatment process

	Anaerobic digestion biosolids		Aerobic digestion biosolids	
	Mass balance	**Calibration Curve**	**Mass balance**	**Calibration Curve**
(Barbarick and Ippolito, 2000)	-	25 – 32 %	-	-
(Gilmour et al., 2003)	-	27 %	-	37%
(Eldridge et al., 2008)			54 %	
(Pu et al., 2008)	43 %	-	59 %	-
(Rigby et al., 2010)	-	39.4 %	-	-

For example, rate of mineralization in the first year in the case of aerobically digested biosolids, were 52 % (Pu et al., 2008) and 53% (Eldridge et al., 2008) in Queensland and New South Wales state espectively, higher than the rate as compared the mineralisation rate for aerobically digested biosolids of 34 – 58 % (mean 47 %).

The first year mineralisation rates are much greater than the suggested 15 % and 25 % organic N for anaerobic and aerobically digested biosolids as stated in the Australian State guidelines (EPA VIC, 2004). The warm soil temperatures and high soil moisture prevalent in regions of Queensland may be responsible for the high mineralisation rates, which are greater than those recorded in temperate regions (Smith and Durham, 2002, Morris et al., 2003). Therefore, further research is required to accurately estimate the N rates of mineralisation for different soil and biosolids types, typical to all states and climatic conditions found in Australia.

2.14 Conclusion

The focus of this chapter was to identify the quantitative data related to the rate and extent at N availability in soil, crop uptake, and N transformation in the case of biosolids amended agricultural land. This assessment was made to understand the N management in terms of nutrient loading rates to meet the crop requirements

without causing nitrate contamination of groundwater or surface water according to the recommendations given in Australian biosolids guidelines (in particular the Victorian guidelines). Moreover, it is essential to identify the gaps in the literature relating to N management in biosolids amended soils in the context of Victorian Guidelines.

Plants require the mineral forms of N such as NH_4-N and NO_3-N, however, most of the N present in biosolids is organic, 80 – 99 % (Figure 2.2). The fraction of total organic N that can be mineralised into forms NH_4-N and NO_3-N, determines the availability of N in biosolids amended soils. The method of stabilisation, biosolids dewatering issues, soil type and environmental conditions are the key parameters that can vary the mineralization. Figure 2.7 shows the mean and range of mineralisable N values determined through laboratory investigation in the published and grey literature. These data reveal that the mean mineralisable N content of approximately 30 % for anaerobically digested biosolids, is double the first year mineralisable N value of 15 % as stated in the Victorian Guidelines (EPA Victoria, 2004). Similarly, the mean value N mineralisation in the case of aerobically digested biosolids was found to be 43 %, compared to 25 % suggested in the biosolids guidelines. However, in the case of composted biosolids, the mean mineralisable N content was estimated to be close to the value suggested Biosolids Guidelines (10 %). Mineralisable N values for lime treated biosolids were similar to aerobic biosolids (37 %), even though these biosolids are different in terms of the biological stabilisation to which they were subjected (Figure 2.12). Lime treated biosolids and aerobically digested biosolids also showed similar mineralisable N values of 44 % when they were used after thermal drying. Since these materials are heated at high temperatures, the organic content of these biosolids undergo structural changes.

Thermophilic aerobic/anaerobically digested biosolids and mesophilic anaerobically digested biosolids both have been shown similar mineralisable N contents of 33%. The rate of N mineralisation (k) in biosolids follows the order as mentioned below

Thermally dried biosolids > aerobically digested biosolids > Lime treated biosolids > anaerobically digested biosolids,

Studies in Australia have demonstrated that thermally dried and aerobically digested biosolids show rapid N mineralisation in comparison to anaerobically digested

biosolids because these biosolids are subjected to extensive stabilisation during the treatment process. Moreover, the storage and drying methods of biosolids have a significant impact on the rate of mineralisation. Lagoon stored and air-dried biosolids contain smaller mineralisable N content as a result of degradation, stabilisation of organic N and ammonia losses through volatilisation due to the long storage period (Gilmour et al., 2003, Cogger et al., 2006). In addition, soil texture, moisture and temperature also vary the rates of N mineralisation in biosolids. The role of soil type on mineralisation rate is not as simple as in other cases. Generally, biosolids containing high organic content have a higher rate of N mineralisation due to it is active microbial population (Rigby et al., 2009). Soils which are better aerated also can increase the mineralisation rate and the best example is coarser sandy soil. However, a high moisture contents favour denitrification due to anaerobic conditions.

Another notable parameter is the specific properties of soil-biosolids blends that also control the nitrogen availability in soil. Combination of less stabilised biosolids types in a fine clay soils blend was reported to promote denitrification (Rigby, 2008, Pritchard et al., 2010).

Inorganic forms of N and mineralised organic N present in the biosolids play a key role in crop uptake of N in biosolids. The proportion of organic-N contained in biosolids reported in the literature can be obtained by the N equivalency method or N mass balance. In the first year of application, mineralisable N values calculated from both field and laboratory data were similar and this reveals that the majority of the mineralisable fraction of organic N is available for crop uptake in the first year after application. In contrast, there are several studies which were conducted to study the second year mineralisation of biosolids N (Cogger et al., 1999, Cogger et al., 2004). However, these values are highly dependent on climatic and seasonal variations, for instance the proportion of mineralisable N that is released in the first year will increase with higher temperature and moisture.

Investigations in Queensland, New South Wales and Western Australia showed high first year mineralisable N values of 57 – 60 %, 45 – 53 % and 39 – 65 % respectively. In Queensland, during the first 7 weeks after biosolids application, 42 – 58 % organic N was rapidly mineralised. After the application of biosolids, several years of monitoring is required to estimate the crop responses to biosolids N due to residual mineral N rather than continued mineralisation of organic N. In NSW, the

rate of mineralisation estimated over the period 1 year in the field studies wthan the potentially mineralisable N fraction calculated from the laboratory incubation studies (Eldridge et al., 2008). All these results clearly support the fact that most of the potentially mineralisable N (93 %) was mineralised during the first year, and the major fraction was mineralised within the first two months. The rates of mineralisation estimated in different field trials conducted in Western Australia, were found to be close to mineralisation rates determined in a laboratory incubation study (Rigby et al., 2009). Such high mineralisation rates during the first year especially in Western Australia and Queensland may be due to the warm climate and high rainfall that causes rapid mineralisation rates. However, rapid N mineralisation rates (35 – 60 %) were observed even in cooler temperatures in a laboratory incubation study conducted at 12.5 $^{\circ}$C, especially to mimic Tasmanian climate (Ives et al., 2010) that is known to have cold climate .

There is limited information available for first year N mineralisation rates in the case of Victorian soils and Victorian climate conditions. However the comparison of the mean N mineralisation values reported in the field and laboratory incubation experiments were found to be different. These discrepancies indicate that EPA Victoria recommendations may significantly underestimate mineralisable N for aerobic and anaerobically digested biosolids although the estimate for composted biosolids may be accurate (Table 2.5). More detailed research is required to determine the suitable application rates.

Table 2.5 Mineralisable N reported in the literature compared to the recommended first year mineralisation recommended by EPA Victoria (2004)

	EPA Victoria	Literature mean
Aerobically digested	25%	45%
Anaerobically digested	15%	32%
Composted	10%	8%

Current N mineralisation rates were derived from overseas data and do not take into account the Victorian environmental conditions and may under or overestimate the N mineralisation rate of biosolids. Derivation of site specific N mineralisation rate of various biosolids amended on different soil types needs to be considered. Hence,

there is a significant research gap with regard to quantifying the mineralisation rates of organic N and PAN contained in different biosolids amended in various soil types under Victorian environmental conditions.

3

3 MATERIALS AND METHODS

3.1 Introduction

This chapter describes the laboratory incubation and field plot experimental designs, the different analytical techniques utilized to quantify the various physicochemical properties of soils, biosolids, and biosolids amended soils, microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) and plant tissue. It also describes the soil, biosolids and plant sampling procedures used during the project.

The field experiments were conducted on two sites, one at Melton Recycled Water Plant (MRWP) at Surbiton Park, South Melton with clay loam soil (Red Sodosol) and the other site at Lara (LA) with sandy loam soil (Brown Sodosol).

At each of the two sites, separate experiments were established. Laboratory incubation experiments were also carried out to investigate the mineral N, MBC and MBN dynamics. Two different soil types were amended with two biosolids types and two fertilizer types (urea and ammonium chloride) under laboratory conditions of temperature 25 °C and moisture of 40 % water holding capacity (WHC). Soil samples were incubated for 91 days at different time intervals. Samples were removed from the incubator and analysed for NO_3-N, NO_2-N, NH_4-N, and microbial biomass (C&N). Full details of the experimental procedures are provided in Chapter 4 (Section 4.2). A second set of experiment were established to investigate the N transformation process under field conditions using the same two biosolids types and fertilizers, each at one application rate with and without plants. A randomised complete block design RCBD was used for all treatments and conducted in triplicate. The details of the experimental procedures for the field experiment are presented in

Chapter 5 and 6. A the third set of experiments focused on determining the mineralisable organic N using nitrogen calibration plots where two biosolids and urea were applied at increasing rates (five rates and a control plot) based on total N loading rate. For this, a systematic design was used with six treatments each in triplicate arranged in RCBD using ryegrass as an indicator crop to quantify plant available nitrogen (PAN). Details of the experimental procedures are shown in Chapter 7 (Section 7.2).

3.2 General description of the study area

3.2.1 Melton Recycled Water Plant (MRWP) at Surbiton Park, South Melton

The shire of Melton is located in Victoria (approximately 19 km west of Melbourne). The shire is located within a dry temperate climate region that experiences considerable winds and variation in temperatures (Melton Environmental Atlas Draft ,2007). In addition, it is positioned within the rain shadow of the Otway Ranges so rainfall is low and erratic, averaging between 465-600 mm per year. The hilly northern part of the shire receives higher rainfall. The combination of spring rainfall and few frosts, makes the area a favourable environment for growing crops. Higher rainfall in the north is also suitable for forestry plantations, which currently exist in the northwest of the area (Melton Environmental Atlas Draft ,2007). The average temperatures are between 7 – 10 °C in the northern and the southeast of the Shire. The average annual maximum temperatures range between 18 – 20 °C around Melton and the southeast of the shire.

Melton's grassy plains and grassy woodlands were once dominated by kangaroo and wallaby grasses. The major farming practice in this area includes growing oats, barley, wheat and canola as winter crops, in some cases integrated with sheep farming using alfalfa. The soils are typically red Sodosols in the Melton Southern district (Melton Environmental Atlas Draft ,2007).

3.2.2 Lara (LA)

Lara is in the shire of Corio approximately 18 km north-east of Geelong, Victoria. Hovell's Creek runs through Lara and flows into Lime Burners Bay, a small inlet of Corio Bay. The region is the driest in Southern Victoria because of the Otway Ranges' rain shadow resulting in only about about 425 millimeters of rain per year.

Furthermore, as the creek is basically ephemeral it is not useful as a water source (Wilkinson, 1972). Granite peaks known as the You Yangs, rise dramatically out of the flat landscape to a height of 352 meters and can be seen from most areas of Geelong.The land surface has been eroded for millions of years since the granite was exposed. However, as granite is a hard rock, it has resisted erosion better than the surrounding rocks. The size and shape of the rounded peaks were caused by fractures in the granite as a result of slight shrinkage episodes during cooling. Weathering and erosion of the granite has resulted in the formation of a blanket of sandy soil (Brown Sodosol) that covers with surrounding rocks (McHaffie and Buckley, 1995). Because the rainfall is low, the vegetation is grassland or low woodland. Small and intermittent salt production has been recorded from a number of small natural salt lake deposits in the far west of the region. In winter these lakes are partially filled by rising groundwater that is rich in salts. The salts are usually precipitated in summer as the water evaporates. Salt is collected both in its naturally occurring form directly from the lake bed as well as from specially prepared crystallizing areas where salt from the lake, dissolved by winter rains, is recrystallised during spring and summer (Bush et al., 1995b).

3.2.3 Selection of biosolids

The anaerobically digested dewatered biosolids (ANDB) used for both laboratory and field experiments were generated from Melton Recycled Water Plant (MRWP) which was classified as C2/T3 (contaminant grade 2 and treatment grade 3) according to the Victorian EPA classification system for biosolids (EPA, VIC ,2004). These biosolids were produced through anaerobic mesophilic digestion at 35 °C for approximately 15 – 30 days prior to dewatering. The dewatered biosolids are sent off-site (3 months) for composting and then sent on to markets.

The aerobically digested dewatered biosolids (ADB) produced at Barwon Water (BW) were stockpiled for 3 years at Western Treatment Plant Werribee. These biosolids were classified as C2 / T2 by EPA Victoria (EPA, VIC ,2004). Samples of both biosolids types were homogenised thoroughly, transferred into plastic bags and transported to laboratory. Sub-samples were stored at 4 °C while the rest of the biosolids were air dried for two weeks and sieved to pass < 2 mm mesh.

3.2.4 The experimental sites

The two rectangular field sites selected were each 120 m × 20 m. Both sites had different soil types, with low background nutrient concentrations. These sites had never previously received biosolids. The first site, having a clay loam soil, was located at the MRWP, Surbiton Park, South Melton (37° 45.0' 7.99"S 144°35' 18.54"E). The mean temperature and total rainfall during the growing period in this site were 15.6 °C and 237 mm respectively. The second site, having a sandy loam soil, was located at LA, (37° 57'490.5"S 144° 30.0' 1.73"E). The mean temperature and total rainfall were 16.4 °C and 240.4 mm respectively. Perennial ryegrass (*Lolium perenne*) was used as an indicator crop, as it is commonly grown as a pasture crop and is very efficient in taking up nutrients.

3.2.5 Field Experimental Design and Treatments

Two separate experiments, the first one to quantify the plant available nitrogen using N calibration. The second one to investigate N dynamics and quantify the mineralisable N in each of the sites was established. The first experiment with a size of 38 m × 11.5 m was set aside to establish the N calibration plots where plant response to increasing amounts of biosolids and conventional fertiliser application based on total N were examined. The calibration curve was then used to estimate the plant available N from the biosolids, by direct comparison of the response from a second set of perennial ryegrass plots which had received urea at increasing application rates.

The second experiment was conducted to examine the dynamics of N and quantify the mineralization rate of N under uncontrolled conditions (Experiments 2). Results of this experiment were contrasted with the findings of the laboratory incubation experiment which was conducted under temperature and moisture controlled conditions.

Before setting up the field experiments, thirty cores of soil from each of the sites were randomly taken at a 10 cm soil depth using an auger (9 cm diameter, Ergonomic hand auger set. Eijkelkamp). The samples were combined, homogenized and transferred to plastic bags and transport to the laboratory. Sub-samples were taken and stored at 4°C, while the rest of the soil was air dried for two weeks and sieved to pass through a < 2 mm mesh for further analysis.

The experimental sites were fenced and the herbicide glyphosate (at a rate of 3L

Figure 3.1. Applying the herbicide glyphosate and mowing the site

/100L water) was sprayed on the experimental site to eradicate the weeds, and the grass was mowed, raked and cleared (Figure 3.1)

Each site was ploughed using a tractor and then rotary hoed (Figure 3.2) and further divided into the two separate experiments described above; each experiment was partitioned as main plots and plots as per the design for each of the experiments.

Figure 3.2. Ploughing the plots prior to partitioning the site at Melton Recycled Water Plant

Approximately 1.5 tonnes of anaerobically digested dewatered biosolids generated from MRWP (at Surbiton Park site) were transported to both sites.

Aerobically digested dewatered biosolids (2.5 tonnes) sourced from Barwon Water was transported from the Western Treatment Plant at Werribee to both sites (Figure 3.3).

Figure 3.3. Transportation of biosolids to the experimental sites

3.3 Measurement of physicochemical properties of soil and biosolids

3.3.1 pH and electrical conductivity (EC)

The pH_w and pH_{ca} of soil and biosolids samples were determined, each in triplicate according to Method 4A1 and 4B2 (Rayment and Lyons, 2011) respectively, using ratio of 1:5 soil/water suspension. Air-dried soil samples (20 g) were sieved to < 2 mm and transferred to plastic bottles and 100 mL of Milli Q water was added using a measuring cylinder. Samples were shaken for 1 hour at 130 rpm using platform mix shaker and allowed to settle for 30 minutes. The pH meter (Hanna pH 211, Micropcssor pH meter) was calibrated using pH_4 and pH_7 buffer solutions. After measuring $pH_{w,}$ 1mL of 1*M* $CaCl_2$ was added using a micropipette to each soil solutions and shaken for further 30 minutes, and allowed to settle for 30 minutes then the pH measured

Electrical conductivity (EC) of samples of soil solutions was measured following the Method 3A1 (Rayment and Lyons, 2011) using a 1:5 soil/water extract.

Air-dried soil samples (20 g) were sieved < 2 mm weighed and transferred into plastic bottles. Milli Q water (100 mL) was added to each bottle, shaken for 1 h and allowed to settle for 30 min. The ECONSCAN conductivity meter was calibrated using 0.01 *M* KCl at 25 $^{\circ}$C as a reference solution and data were recoded for EC in µS/cm.

3.3.2 Measurement of Soil Bulk Density

From each experimental site, a cylinder of known dimensions was hammered into the soil and carefully removed to collect the whole soil sample for bulk density measurements (McIntyre and Loveday, 1974). The fresh mass of each soil sample was recorded and the soil dried to constant mass at 105 $^{\circ}$C. A dry bulk density of each soil type was calculated as follows:

Bulk density (g cm^{-3}) = total dry mass of the sample (g) / volume of cylinder (cm^3).

3.3.3 Determination of organic matter in soil

Organic matter affects many physical, chemical and biological soil properties including soil structure, soil compressibility and shear strength (Ray and Fred, 2004). In addition, it affects the nutrient contributions, water holding capacity, biological

activity and water and air infiltration rates (Six et al., 2000). This examination is performed to determine the organic matter in the soil as a ratio, and then expressed as a percentage of mass of organic matter in a given mass of soil/biosolids to the mass of the dry soil/biosolids. The content of organic matter (OM) in soil and biosolids was measured using the loss on ignition technique as per Method 6G1 (Rayment and Lyons, 2011). A 5g sample of air-dried soil was selected and the moisture content measured as described in Section 3.4.4. The porcelain dish mass (M_P) was recorded. Subsequently samples were weighed into the dish and then the combined mass of dry soil and porcelain were recorded (M_{PDS}). The dish was placed into the muffle furnace and gradually the temperature was increased to 550^{o}C. The porcelain dish was left in the furnace overnight (24 h). The dish was then cooled in a desiccator and the mass of the porcelain dish containing the ash (burned soil) (M_{PA}) was recorded.

Data analysis was calculated as following:

The mass of the dry soil (M_D) = $M_{PDS} – M_P$

The mass of the ashed soil (M_A) = $M_{PA} – M_P$

The mass of organic matter (M_O) = $M_D – M_A$

Organic matter content (OM %) = $M_O / M_D \times 100$

3.3.4 Gravimetric Moisture Content (%)

The moisture content of soil and biosolids samples were determined as per Method 2A1 (Rayment and Lyons, 2011). Sub-samples (10g) of soil and biosolids were air-dried and sieved to < 2 mm. Samples were dried at 105^{o}C overnight and then placed in desiccators and allowed to cool. The moisture content was calculated from the weight loss.

3.4 Soil and biosolids analysis for nutrients and heavy metals

3.4.1 Determination of total nitrogen (TN)

Samples (0.5 g) of soils, biosolids and plants were weighed in triplicate and the total N was determined using a Leco FP 2000 Carbon and Nitrogen Auto 77olybdit in the University of Melbourne, Creswick Campus

Australian soil and plant analysis council (ASPAC) standard reference materials (STD 75, STD 55 and Compost 5 for soil and biosolids) and ASPAC standard reference materials STD 143 tea leaves and STD 63 eucalyptus leaves were also analysed for TN concentrations to validate the accuracy of soil and plant analytical results.

3.4.2 Measurement of total oxidized N (NO_3-N+ NO_2-N)

The automated spectrophotometric method (flow injection analysis, FIA) was used to determine nitrate-N in soil and biosolids samples. In this method, nitrates are reduced to nitrite by a copper cadmium reductor coil (CRC). The nitrite ions react with 78olybdite78mide under acidic conditions to form a diazo compound. This couples with N-1-naphthyl ethylenediamine dihydrochloride to form a reddish purple azo dye (corporation, 1971).

Soil and biosolids were extracted using 1 *M* KCl extracting solution to analysis NO_3-N(Keeney and Nelson, 1982). The extraction solution was prepared by dissolving 47.55 g of potassium chloride (KCl) in 1000 mL of Milli- Q water. A 5 g sample of soil or biosolids were weighed into 100 mL plastic bottles and 50 mL of 1 *M* KCl extraction solution was added (ratio 1:10). A blank was included (50mL of 1 *M* KCl extraction solution). Samples were shaken using a platform mixer shaker for 30 minutes at a speed of 160 rpm, and filtered using a Whatman number 42-filter paper (150 cm) and stored in the dark at 40C.

The following reagents, calibration standard solution and buffer solution were prepared:

Sulphanilamide ($C_6H_8N_2O$)

Sulphanilamide (5 g) (Analytical grade, Scharlau) was dissolved in a mixture of 26 mL concentrated HCl and 300 mL of Milli-Q water added to a 500 mL volumetric flask. This was diluted to the final mark with Milli-Q water and sonicated for 30 minutes to remove air bubbles.

NED dihydrochloride solution (C12H14N2.HCl)

N-(1-Naphthyl) ethylenediamine dihydrochloride (NED dihydrochloride, Sigma) (0.5 g) of (Analytical grade) was dissolved in 500 mL Milli-Q water and sonicated for 30 minutes.

Buffer NH_4Cl solution

NH_4Cl (85 g) and Na_2EDTA (3 g) of were dissolved in 920 g of Milli-Q water to prepare the buffer solution. The pH of the solution was adjusted to 8.5 by adding 5 – 6 mL concentrated ammonia (28%), and then diluted with Milli-Q water.

Preparation of copperized-cadmium reduction column

A 2% copper sulphate solution was prepared by dissolving 10 g $CuSO_4.5H_2O$ in 500 mL of Milli-Q water. Cadmium granules (10 g) were weighed and swirled in 6 M HCl for 1 minute to remove any oxide coating after which the supernatant was decanted and the cadmium granules were rinsed with Milli-Q water. The granules were then swirled in portions of 2 % copper sulphate for 5 minutes and the supernatant was decanted. This process was repeated until a brown colloidal precipitate formed; finally the granules were washed with Milli-Q water and rinsed with NH_4Cl buffer solution.

Preparation of stock NO_3-N solution (100 mg/L NO_3-N)

Analytical grade sodium nitrate ($NaNO_3$) (0.3035 g) of was dissolved in 500 mL of Milli-Q water. A 20 mL aliquot of this 100 mgL^{-1} stock solution was diluted with Milli-Q water in a 100 mL volumetric flask to make the intermediate stock standard solution.

Calibration standards were prepared by taking a series of volumes (0, 1, 2, 4, and 8 mL) of the intermediate stock solution and diluted to 100 mL with Milli-Q water which were equivalent to 0, 0.2, 0.4, 0.8 and 1.6 mgL^{-1} NO_3-N.

Samples, standards, and blanks were analysed using the cadmium-reduction technique in a flow injection analyser with the absorbance of the reddish purple colour measured at a wavelength 640 nm. When the concentrations exceeded the calibration range, soil and biosolids extracts were diluted with the extraction solutions and reanalysed.

The portable flow injection analyser instrument which was manufactured by Monash

University (Water Studies Centre, School of Chemistry) was used in this study for analysis of nitrate in soil and biosolids extracts.

Validating the Analytical data

There are no standard reference samples for accurate measurement of nitrate. Precision measurements for NO_3-N carried out for soil test quality assurance program of the Alberta Institute of Pedology (Heaney et al., 1988) indicated that NO_3-N was one of the most variable parameters determined. To validate the analytical data, extracts were spiked with 1mL of the 1.6 mg/L NO_3-N standard reanalysed. The percentage recovery was calculated and found to be between 95 to 105%.

3.4.3 Determination of ammonium (NH_4-N)

The automated phenate method was used to analyse NH_4-N in 1 *M* KCl extract solution. This method is based on the automated colourimetric method documented in the APHA-AWWA-WPCF (2005) "Standard Methods for the Examination of Water and Wastewater", but makes use of a flow injection analyser, instead of an auto analyser. Ammonia, in the sample, reacts with hypochlorite to form monochloramine which, in the presence of phenol, nitroprusside and excess hypochlorite, gives indophenol blue (Paul Armishaw., 2002). The result is the formation of indophenol blue, which is proportional to the concentration of ammonia, and can be measured spectrophotometrically at 630 nm. High concentrations of calcium and magnesium ions will cause precipitation during analysis. The use of EDTA eliminated the problem. The samples were prepared to determine NO_3-N concentration. The rest of each 1 *M* KCl extraction solution kept at -19^{o}C. All samples sent to Monash University to analysis the concentration of NH_4-N. The analysis completed within two weeks' time.

3.4.4 Determination of Olsen-P

The method of Olsen *et al*., (1954) has wide international acceptance as an indicator of soil P fertility. Olsen's method is based on extraction of air-dried soil with 0.5 M $NaHCO_3$ and adjusted to pH 8.5 with NaOH. Soil extraction is for 30 minutes at a soil/solution ratio of 1:20 as per Method 9C1 (Rayment and Lyons, 2011). The following reagents and standard solutions were prepared for the procedure:

Stock Extracting solution (0.5 M $NaHCO_3$)

A 42 g of sodium bicarbonate was dissolved in 900 mL Milli-Q water and 0.8 g NaOH was added to adjust the pH to 8.5 and diluted to 1000 mL with Milli-Q water.

Stock Ammonium Molybdate solution (R1)

A 12g of ammonium 81olybdite was transferred to a 500 mL volumetric flask and then 250 mL of Milli-Q water was added and sonicated until completely dissolved.

Stock Potassium antimonyl tartrate solution (R2)

The potassium antimomnyl tartrate was prepared by weighing 0.2908 g in 100 mL of ultra-pure water.

Sulphuric acid (2.5 M) (R3)

Milli-Q water was added into 1000 mL volumetric flask and then 139 mL of sulphuric acid (18 M H_2so_4) added to the volumetric flask carefully under Fume hood for the purpose of safety.

Reagent A

Stock solution of R1, R2 and R3 were transferred into 2000 mL volumetric flask and diluted with Milli-Q wateruntil final mark of volumetric flask. This solution can be stored for 6 months.

Reagent B

Ascorbic acid was prepared by weighing 1.056 g and diluted with 200 mL of reagent 2. This solution was prepared every day.

Indictor solution

A 0.5 g of 4-p-Nitrophenol was dissolved with 25 mL of ultra-pure water.

Standard stock solution (100 mg P/L)

Phosphorous stock solution was prepared by dissolving 0.2197 g of KH_2PO_4 in 0.5 *M* $NaHCO_3$ (pH 8.5) and made up to a final volume of 500 mL.

Intermediate stock solution (20 mg P/L) and Working Calibration Standard solutions

To prepare the intermediate stock solution, 20 mL of the 100 mg P/L stock solution was diluted with extraction solution (0.5 *M* $NaHCO_3$ (pH 8.5) in 100 mL volumetric flask. Working calibration standard solutions were prepared by taking 1, 2, 3,4,5,7.5 and 10 mL of the intermediate stock solution (20 mg P/L) in 100 mL volumetric flasks which were equivalent to 0.2, 0.4, 0.6, 0.8, 1, 1.5 and 2 mg P/L concentrations and diluted to the mark with the extracting solution.

Sample preparation

A 2.5 g of soil and biosolids samples were weighed in 250 mL plastic bottles then 50 mL of the extracting solution was added, shaken for 30 minutes and filtered using Whatman number 42, filter paper (150cm).

The UV-Visible spectrophotometer (Cary 50 Bio) used to analysis P in soil and biosolids extracts. The absorbance of the blue complex was recorded at 820 nm.

Soil and biosolids extracts were analysed in triplicate by diluting them with extraction solution in a 25 mL volumetric flask.

Validating the Analytical data

The samples were validated by spiking with known amounts of phosphorous and the percentage recoveries were calculated which ranged from 90 to 110%.

3.4.5 Determination of total phosphorus (TP) and heavy metals

Total metals in soil, biosolids and biosolids amended soil samples were analysed using a Bruker S4 pioneer (Bruker AXS, Karlsruche, West Germany) wave length dispersion- Xray fluorescence spectrometry, equipped with LiF, LiF (200), Ge, PET, OVO -55 crystals with a detection limit ranging between 10-100 $\mu g g^{-1}$ for soil (Schlotz and Uhlig, 2002).

X-ray fluorescence analysis was carried out by weighing 8 g of each of samples and 2 multimixes XRF pelleting tablets (XRF, multi-mix, PXR-250, Premier) (each weighing 1 g were added in each of the samples and ground using a Zirconium made Lab Technics Ring Mill. The fine particulate samples were transferred into an aluminium cup and packed using an Enerpac hydraulic pressure packer. The pressed pellets were analysed in triplicate.

For XRF analysis of total metals in soil/biosolids and biosolids amended soils samples, external calibration curves for each of the metals ((Cu, Cd, Co, Cr, Mo, Mn, Fe, Pb, Ni, Zn,) and major cations (Na, K, Ca, Mg, Al) were established by analysing eight soil standard reference materials (NCS DC 73319, NCS DC 73320, NCS DC 73321, NCS DC 73322, NCS DC 73323, NCSDC 73324 NCS DC 73325 NCS DC 73326). To validate the established calibration curves, Till 1 and Till 3 soil standard reference materials (Canadian Certified Reference, Materials Project CANMET Mining and mineral sciences Laboratories, ON, Canada, 2005) were treated as

samples and analysed based on the already established calibration curves and the precent recovery was calculated.

3.5 Measurement of soil microbial biomass

3.5.1 Chloroform Fumigation-Extraction Procedure

A chloroform ($CHCl_3$)-fumigation, direct extraction (CFE) protocol based on the methods of (Saggar et al., 1981, Brookes et al., 1982, Brookes et al., 1985a, Brookes et al., 1985b, Vance et al., 1987a) was used for measurement of microbial biomass C (MBC) and microbial biomass N (MBN) concentrations in soil and biosolids treated soil. There are many advantages with this method as it is rapid, and is valid to soil with low pH (Couteau and Henkinet, 1990) and for soils treated with organic substrates of (Vance et al., 1987a). The principle of the CFE technique is exposing the soil to $CHCl_3$ vapors which kills all microorganisms' cells releasing soluble extractable components, which can then be determined as an indication of the amount of total microbial biomass in soil.

3.5.2 Soil preparation

Biosolids amended soil samples with 40 % (water holding capacity) were frozen at -19 $^{\circ}$C, to keep microorganisms inactive. The samples were taken out from freezer and then left for 6 h to defrost. Triplicate 30 g samples of each soil were weighed into glass containers. Moist paper towels were placed in the desiccator to maintain humidity during the fumigation to avoid surface dryness of the samples.

50 mL of amylene stabilized $CHCl_3$ was transferred into 100 mL beaker and then anti-bumping granules were added. The beaker and was placed in the middle of the desiccator along with the samples. The fumigation chamber was closed and evacuated for 1 – 2 min until the $CHCl_3$ boiled vigorously. The sealed desiccator was kept in the dark for 24 h at 25 $^{\circ}$C. After the incubation period (overnight), the vacuum was released, the paper towels were removed, and the fumigation chamber was evacuated several times to remove residual of $CHCl_3$. Each evacuation was for 2 minutes and air was allowed into the fumigation chamber after each evacuation to remove residual of chloroform.

Extraction solution (0.5 M K_2SO_4)

The 0.5 *M* K_2SO_4 extraction solution was prepared as follows. 87.13 g of analytical grade of potassium sulphate (K_2SO_4) was dissolved into 950 mL ultra-pure water. The solution was stirred on a magnetic stirrer until the salt completely dissolved (approximately 2h). The solution was then adjusted to 1000 mL with deionised water.

A 5:1 ratio of extracting solution to sample was used by weighing 10 g of fumigated samples into 250 mL plastic bottle and 50 mL of 0.5 *M* K_2SO_4 added (Horwath and Paul, 1994). Non-fumigated samples were extracted at the same time, following the same procedure. A blank sample of 50 mL (0.5 *M*) K_2SO_4 was included with each batch. The samples were shaken for 1hr and then filtered using Whatman Number 42 (150 mm) filter papers and transferred into 250 mL plastic bottles.

3.5.3 Microbial biomass Carbon (MBC)

The analysis of the total organic carbon (TOC) was based on wet chemical oxidation, using the Non-Purgeable Organic Carbon (NPOC) method. This procedure is recommended for soil analysis because it is prone to less error than the alternative Total Carbon-Inorganic Carbon (TC – IC) method (Shimadzu, 2003).

The previously filtered extracts which were kept at -19 ^{o}C until analysis for TOC and, were taken out from the freezer and allowed to cool to room temperature. The extracts were transferred into 40 mL glass vials, covered with parafilm to prevent atmospheric C contamination, and were placed in the automatic rack for analysis. Analysis of the samples was completed over approximately n two weeks'.

The MBC concentration was calculated according to the following equation:

$$\textit{Microbial biomass C } (\mu g\ C\ g^{-1}\ ds) = (C_F - C_{UF}) / K_{EC}$$

Where $C_F - C_{UF}$ is the soluble organic C extracted from fumigated soils minus that extracted from non-fumigated soil and K_{EC} = 0.35, is referred to the efficiency of extraction of MBC. This value ranged from 0.25 to 0.45 (Wu. J. et al., 1990,Joergensen, 1995, Joergensen and Mueller, 1996b).

3.5.4 Microbial biomass Nitrogen (MBN)

The following stock solutions were prepared to use in this study:

3.75 M NaOH solution

A 150 g of NaOH pellets dissolved in 900 mL of Milli-Q water and then let the solution cool down until room temperature and adjust the volume by adding Milli-Q water to 1000 mL.

Persulphate digestion

Persulphate oxidizes and converts dissolved organic N (DON) to NO_3-N. In this study, the cadmium reduction method was used to determine the microbial biomass N.

To prepare the stock solution, 100 g of peroxydisufate ($K_2S_2O_8$) was weighed and transferred to a 1000 mL beaker, and 200 mL of 3.75 *M* NaOH and 60 g of boric acid (H_3BO_3) was added to the beaker. The beaker was placed on a magnetic stirrer until the salt completely dissolved and then final volume of 2000 mL adjusted by adding Milli-Q water.

Nicotinic Acid (100 mg /L $C_6H_5NO_2$)

100 mg /L $C_6H_5NO_2$ was prepared by dissolving 0.43968 g of Nicotinic acid in 500 mL Milli-Q water.

Oxidation Procedure

15 mL of 0.5 *M* K_2SO_4 extract samples were transferred into 50 mL centrifuge tube. A 15 mL of the oxidative solution which involves 3.75 M NaOH, peroxydisufate ($K_2S_2O_8$) and boric acid (H_3BO_3) was added in each tube. The extraction solution was prepared as described in Section 3.6.2 to make calibration equation treated as samples and also including the blank standard (15 mL of oxidative solution). To measure the efficiency of the analytical procedures, 10 non-fumigated samples were spiked by adding 5 mL of nicotinic acid (20 mg /L) and the recovery of organic N in nicotinic acid was calculated. The cap of centrifuge tube was left on the top without closed very well because it may explode. For a few second, the tubes were agitated on a vortex for several minutes to ensure that mix well. Each tube was weighed and all tube samples including the calibration standard and blank were placed in the autoclave and the temperature gradually increased to 121 ^{o}C (135 Kpa). After autoclaving, samples were allowed to cool down to room temperature and then reweighed to calculate mass loss. Then Milli-Q water was added to each centrifuge tube to make a final volume (40 mL). The solutions were then transferred

quantitatively into 100 mL volumetric flasks rinsing with 0.5 *M* K_2SO_4 several times and then diluted with 5 *M* K_2SO_4 to the mark.

The samples were analysed using the cadmium reduction technique. The concentrations of MBN was estimated as shown below (Brookes et al., 1985b):

$MBN\ (\mu g\ N\ g^{-1}\ soil) = (N_F - N_{UF}) / k_{EN}$

Where N_F and N_{UF} is the amount of N extracted from $CHCl_3$-fumigated – non-fumigated samples and k_{EN} = 0.54 was used as the efficiency of extraction of MBN and the values ranged from 0.18 to 0.54 (Joergensen and Mueller, 1996a).

3.6 Measurement of total nitrogen (Nt) in Ryegrass samples

3.6.1 Samples preparation

Plant sampling was conducted 120 days after sowing from both experimental sites.

The samples were harvested from 1 m × 1 m quadrants from the centre of each of the subplots leaving 2 cm above the ground. The samples were transferred into paper bags and transported to the laboratory where fresh biomass was recorded. There was a time delay of between 3 – 6 hours from the time of harvest to the time of measurement. The samples were then dried at 65 $^{\circ}$C for 48 hours in a fan-forced oven (Thermoline Scientific, TO-500F).

3.6.2 Total nitrogen in Ryegrass samples

Sub-samples of dry biomass were ground to a fine powder using a ring mill (Lab Technic Model LM 1/P). Sub-samples of the powdered plant material were analysed for total C and N using the Leco CHN 2000 auto carbon and nitrogen analyser. All analyses were performed by the Laboratory at University of Melbourne (School of Land and Environment, Department of Forest and Ecosystem Science, Creswick).

3.7 Statistical procedures

Plant biomass, soil, biosolids and environmental data collected from the two field experimental sites were subjected to a two way analysis of variance (ANOVA) including biosolids and block effects. Minitab version 16 statistical software was used for all of statistical analyses. Analytical data generated from laboratory experiments were also statistically analysed using a two way ANOVA and regression procedures.

A two sample t-test was also conducted to check whether there were significant differences between biosolids and soil types. The ratio of the regression coefficients of the biosolids curve to the regression coefficient of the urea treated calibration curve was used to quantify the plant available N values of the two biosolids types.

4

4 A Laboratory Investigation of the Mineralisation Rate of Nitrogen and Immobilisation of Carbon and Nitrogen in Fertiliser and Biosolids Amended Soil

4.1 Introduction

The quantities of N in biosolids that can become available to crops during the growing season should be known so application rates can be adjusted to provide crops with sufficient N without causing nitrate contamination of surface or ground water.

Soil incubation studies have been extensively used to determine N availability in organic soil amendments (Tester et al., 1977, Hsieh et al., 1981, Parker and Sommers, 1983, Smith et al., 1998b, Smith et al., 1998c, Breedon et al., 2003). They allow the rates of mineralisation of organic N amendments to be quantified without interferences due to losses of N by crop uptake or leaching through the soil profile.

Various factors influence the N mineralisation rate including soil moisture, soil temperature, pH, soil types and biosolids types (Chapter 2, Section 2.6). Another potential factor is the microbial biomass which also varies with soil types.

The quantities of N mineralised from organic matter are a balance between total mineralisation and immobilisation. The extent of immobilisation is mainly dependent on the C: N ratio and structure of the organic matter (Sabey et al., 1975,Nicolardot et al., 2001, Vinten et al., 2002, Pansu and Thuriès, 2003). Microbial biomass C and N dynamics have previously been investigated in biosolids-amended soil under both laboratory (Franco-Hernández et al., 2003, Wang et al., 2003a, Hseu and Huang,

2005, Abbasi and Khizar, 2012) and field conditions (Banerjee et al., 1997, Jedidi et al., 2004, Calbrix et al., 2007). Most of the laboratory studies reported that the greatest amount of organic-N was mineralised within 56 days (Smith et al., 1998c).

The main purpose of this work in this chapter was to investigate the rates of mineral N in clay loam and sandy loam soils amended with conventional fertilisers (urea and NH_4Cl) and two biosolids types, under controlled laboratory conditions. More specifically, it was to estimate the rate of mineralisation of the two fertilisers and two biosolids during a three month incubation period. In addition, microbial biomass C and N were measured to determine the amount of N immobilised in the biomass

Following the method of Smith et al., (1998a), a laboratory incubation experiment was set up under controlled temperature (25 ºC) and soil moisture (40 % water holding capacity) throughout the experiment. The rates of N mineralisation and immobilisation of C and N were measured. One application of two biosolids and conventional fertiliser was applied to a clay loam and sandy loam soil. The influence of soil type and biosolids type on mineral N is examined. The biosolids amended soil samples were taken on days 1, 3, 8, 14, 28, 42, 56, 70, 84 and 91 to determine NO_3-N, NH_4-N, MBC and MBN.

4.2 Setting up the experiment

4.2.1 Sampling soil and biosolids

Approximately 30 kg of fresh moist soil samples were collected from MRWP at Surbiton Park and from LA on the 8th of March 2011. A portion (500 g) was air dried and sieved through < 2 mm (10 Mesh), prior to chemical analysis (Figure 4.1). The rest was treated as described in Chapter 3 (Section 3.2). Soil samples were taken from 0 –15 cm depth, because this layer has the greatest microbial activity and soil organic matter (Murphy et al., 1998).

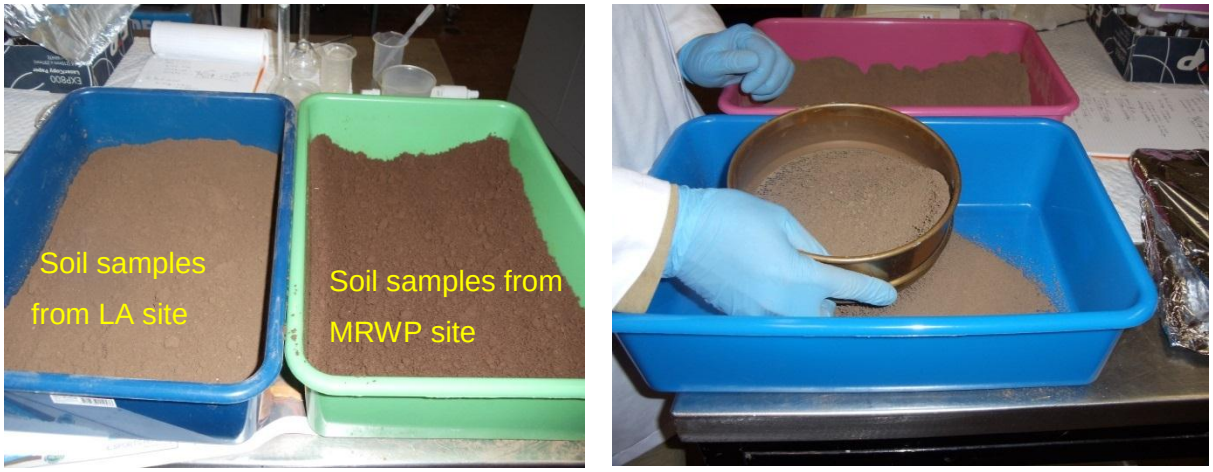

Figure 4.1. The two soil types (3.1 kg) used to set up laboratory incubation experiment

Biosolids from MRWP at Surbiton Park (5 kg) and BW (5 kg) were transported to the laboratory. A proportion of these biosolids (1 kg of each) were air dried for one week and sieved to pass through < 2 mm mesh. The rest was frozen at -19 °C to prevent nitrification of the ammonium-N into nitrate and to keep the microorganisms inactive.

Subsamples of soil and biosolids were analysed using standard analytical techniques (described in Chapter 3, Section 3.4) for characterization of physico-chemical parameters.

4.2.2 Determination of water holding capacity and adjusting soil moisture

The water holding capacity (WHC) of soil was measured using a modification of the method described by Harding and Ross (1964). Samples of approximately 100 g of each soil were placed in a small sieve lined with a Whatman 150 mm filter paper, and placed in a dish of water so that the water level was higher than the level of the soil to allow the soil to become saturated. Samples were left to stand for 8 h to achieve equilibrium. The sieves were then removed from the dish of water, covered by parafilm to avoid evaporation, and left for 3 h to drain.

Triplicate subsamples (20 g) of each saturated soil were weighed into aluminium dishes, recording the weight of the dish before adding the samples. The soil samples were dried overnight at 105 °C to achieve constant weight. The water holding capacity (WHC) was calculated as follows:

WHC (%) = 100 x (mass of drained soil – mass of oven dried soil) / (mass of oven dried soil)

The WHC for soil from the LA site was 42 %; soil samples taken from MRWP were air-dried for 8 h to reduce their moisture content to approximately 44 % (WHC), which is within the standard range used for laboratory incubation experiments (Harding and Ross, 1964, Smith et al., 1998a, Smith et al., 1998b, Smith et al., 1998c).

4.2.3 Experimental Design and treatments

The incubation experiment was established on the 27th of March 2011. Soil and biosolids amended samples in the incubator were arranged in a randomised completely blocked design (Appendix B). There were 10 treatments (2 unamended control soils (one of each soil type) + (2 biosolids types each at one application rate × 2 soil types) + (2 fertilizer types, each of them with one application rate × 2 soil types)). All treatments were replicated 30 times giving a total of 300 samples. The three replicate samples from of each the treatments were taken throughout the incubation period (91days). Each replicate was analysed for NO_3-N, NH_4-N using 1 M KCl extraction, and MBN using the chloroform fumigation method and extracting in 0.5 M K_2SO_4 (more details are provided in Chapter 3, Sections 3.5 and 3.6).

4.2.4 Calculation of biosolids and fertilizer application rates

The measured bulk density of soil from MRWP at Surbiton Park was 1.51 g cm^{-3}, whereas for soil samples taken from Lara site it was 1.46 g cm^{-3}. It was assumed that the biosolids would be incorporated into 15 cm soil depth. The combination of depth and bulk density was used to calculate the required application rates of biosolids and fertilisers (Appendix B).

Based on N requirements for perennial ryegrass (a crop used in later experiments), urea and ammonium chloride were added to be equivalent to a rate 250 kg N ha^{-1} on both soils. Biosolids were added at an application rate of 15 and 33 t ds ha^{-1} based on the NLBAR, which is equivalent to 510 kg N ha^{-1} of ANDB and ADB. Biosolids and commercial fertiliser's application rates are shown in Table 4.1.

Table 4.1 Biosolids and fertiliser application rates used for the incubation experiment

Biosolids application rates				
Treatments	t ds ha^{-1}	kg N ha^{-1}	biosolids added (g) kg $soil^{-1}$	Total N added (mg kg^{-1})
ANDB applied on MRWP soil	15	510	10	340
ANDB applied on LA soil	15	510	10	340
ADB applied on MRWP soil	33	510	22	340
ADB applied on LA soil	33	510	22	340
Fertiliser application rates				
	kg N ha^{-1}		fertiliser added (g) kg $soil^{-1}$	Total N added (mg kg^{-1})
Urea supplied on MRWP soil	250		0.35	169
NH_4Cl supplied on MRWP soil	250		0.65	165
Urea supplied on LA soil	250		0.39	169
NH_4Cl supplied on LA soil	250		0.65	165

The soil, biosolids and fertiliser mixtures were homogenised using a hand mixer (Figure 4.2).

Figure 4.2. A hand mixer used to homogenise the biosolids and fertiliser with both soil types

Soil moisture was maintained at 40 % WHC (Section 4.2.2) for soil samples taken from MRWP and 42 % water holding capacity for soil samples taken from LA site. The final moisture content of all treatments, including the control soils, was adjusted weekly by adding distilled water to incubation tubes after recording the difference in mass from the initial mass.

Approximately 100 g soil samples for each treatment were placed into incubation tubes and weighed before incubation. The total number of tubes (300) containing soil samples were incubated under soil water holding capacity of 40 %, and 25 °C temperature throughout the experiment (Smith et al., 1998a). The experimental units were arranged in the incubator (Figure 4.3).

A small puncture was made on the lids of all incubation tubes to allow access of flow of air (Figure 4.3). Soil samples amended with biosolids and fertiliser were taken from the incubator (Thermoline scientific) after 1, 3, 8, 14, 28, 42, 56, 70, 84 and 91 days.

Figure 4.3. Arrangement of samples in the incubator

Samples were analysed for soil NO_3-N using 1 M KCl extraction solution and the rest were kept at -19 °C and later sent to Monash University for analysis of NH_4-N. Table 4.2 shows the equations of standard calibration curves used to calculate the concentration of NO_3-N from soil extractions. The equations for the standard calibration curves used to calculate the concentration of MBN are shown in Table 4.3.

Table 4.2 Calibration of linear equations for NO_3-N analysis for all treatments

Day	Date of analysis	Slope	Intercept	R^2
1	31 March 2011	482	7.9	0.9998
3 8 14	14 April 2011	408	6.7	0.9993
28	01 May 2011	471	10	0.9998
42	21 May 2011	432	12.2	0.9996
56 70 84	24 June 2011	447	5.45	0.9995
91	27 June 2011	406	12	0.9996

(A fresh setting calibration was made for each day)

Table 4.3 Calibration linear equations for MBN analyses

Day	Date of analysis	Slope	Intercept	R^2
1	01 April 2011	514	13	0.9997
3	08 April 2011	437	2.4	0.9999
8 14	27 April 2011	450	18.5	0.9994
42	07 July 2011	495	6.4	0.9999
56	03 August 2011	519	12	0.9999
70	15 August 2011	329	10.5	0.9995
84 91	04 September 2011	490	7.6	0.9997

(A fresh setting calibration was made for each day)

Validation of results

To validate the soil NO_3-N data, unamended control soil and biosolids-amended soil extracts were spiked with known concentrations of NO_3-N and analysed in triplicate. The recoveries (%) of the spiked samples compared with the un-spiked samples ranged between 90 – 113 % (Table 4.4).

Table 4.4 Calculation of NO_3-N recoveries using Flow injection analysis (cadmium reduction method as described in section 3.6.2)

		Concentrations (mg NO_3-N/ L) measured		
Incubation period (days)	**Treatments**	**Un-spiked sample***	**Spiked sample***	**Recoveries (%)**
1	Unamended control soil	0.6 ± 0.2	0.9 ± 0.2	94
	ANDB amended clay loam soil	0.5 ± 0.1	0.9 ± 0.2	110
3	Unamended control soil	0.6 ± 0.0	0.9 ± 0.0	113
	ANDB amended clay loam soil	0.9 ± 0.0	1.2 ± 0.0	107
8	Unamended control soil	1.0 ± 0.0	1.38 ± 0.0	99
	ANDB amended clay loam soil	0.9 ± 0.0	1.0 ± 0.0	112
14	Unamended control soil	0.3 ±0.0	0.6 ± 0.0	109
	ANDB amended clay loam soil	0.8 ± 0.0	1.1 ± 0.0	108
28	Unamended control soil	0.5 ± 0.0	0.8 ± 0.0	102
	ANDB amended clay loam soil	0.9 ± 0.0	1.1 ± 0.0	95
42	Unamended control soil	0.6 ± 0.0	0.9 ± 0.0	99
	ANDB amended clay loam soil	0.5 ± 0.0	0.9 ± 0.0	103
56	Unamended control soil	0.8 ± 0.0	1.1 ± 0.0	90
	ANDB amended clay loam soil	0.7 ± 0.0	1.0 ± 0.0	98
70	Unamended control soil	0.4 ± 0.0	0.8 ± 0.0	102
	ANDB amended clay loam soil	0.7 ± 0.0	1.0 ± 0.0	96
84	Unamended control soil	0.7 ± 0.0	1.0 ± 0.0	98
	ANDB amended clay loam soil	1.2 ± 0.0	1.6 ± 0.0	112
91	Unamended control soil	0.5 ± 0.0	0.8 ± 0.0	95
	ANDB amended clay loam soil	0.5 ± 0.0	0.7 ± 0.0	95

1 M KCl extracts of soil samples were spiked with 5 mL of the 1.6 mg NO_3-N /L standards in 25 mL volumetric flask (0.32 mg/L NO_3-N)

* Values indicate mean ± sd of triplicate measurements for each treatments.

To measure the amount of immobilised N in microorganisms, the chloroform fumigation extraction method of Jenkinson and Powlson (1976) was used. The

method has been used previously to estimate the microbial biomass N under both aerobic (Ross, 1987, Wu. J. et al., 1990) and anaerobic conditions (Inubushi et al., 1984, Inubushi and Watanabe, 1986, Inubushi and Wada, 1988). In this method, inorganic N is determined before and after fumigation as described in Chapter 3, Section 3.6.

To measure efficiency of the digestion procedure, un-fumigated control soil and ANDB amended clay loam soil K_2SO_4 extracts using 0.5 M were spiked with a known concentration of nicotinic acid ($C_6NH_5O_2$), which is an organic-N source that easily breakdowns during the digestion procedure, and analysed in triplicate. The recoveries (%) of the spiked samples compared with the un-spiked samples were on average between 83 – 108 % for soil samples amended with biosolids and unamended control soil (Table 4.5).

Table 4.5 Measuring efficiency of the digestion procedure

		Concentrations (mg NO_3-N L^{-1}) measured		
Incubation period (days)	Treatments	Un-spiked sample*	Spiked sample*	Recoveries (%)
1	Unamended control soil	0.2 ± 0.0	0.3 ± 0.0	105
	ANDB amended clay loam soil	0.3 ± 0.0	0.4 ± 0.0	96
3	Unamended control soil	0.7 ± 0.0	1.2 ± 0.0	98
	ANDB amended clay loam soil	0.8 ± 0.0	1.0 ± 0.1	100
8	Unamended control soil	0.5 ± 0.0	0.9 ± 0.0	83
	ANDB amended clay loam soil	0.8 ± 0.0	1.2 ± 0.0	93
14	Unamended control soil	0.6 ± 0.0	1.0 ± 0.0	99
	ANDB amended clay loam soil	0.8 ± 0.0	1.2 ± 0.0	86
28	Unamended control soil	1.0 ± 0.0	1.5 ± 0.0	101
	ANDB amended clay loam soil	0.7 ± 0.0	1.2 ± 0.0	106
42	Unamended control soil	1.0 ± 0.0	1.4 ± 0.0	101
	ANDB amended clay loam soil	0.7 ± 0.0	1.2 ± 0.0	108
56	Unamended control soil	1.0 ± 0.0	1.2 ± 0.0	93
	ANDB amended clay loam soil	0.6 ± 0.0	0.8 ± 0.0	108
70	Unamended control soil	0.6 ± 0.0	0.9 ± 0.0	105
	ANDB amended clay loam soil	0.8 ± 0.0	1.0 ± 0.0	104
84	Unamended control soil	0.5 ± 0.0	0.7 ± 0.0	100
	ANDB amended clay loam soil	-	-	-
91	Unamended control soil	0.9 ± 0.0	1.1 ± 0.0	96
	ANDB amended clay loam soil	1.2 ± 0.0	1.4 ± 0.0	98

0.5 *M* K_2SO_4 extraction soil samples were spiked with 5 mL of the 20mg /L nicotinic acid in 50 mL centrifuge tube (4 mg / L) and analysed and the recovery (%) was calculated.

* Values indicate mean ± sd of triplicate measurements for each treatments.

4.2.5 Estimating the fractions of organic-N mineralised

The concentrations of NO_3-N, NO_2-N, NH_4-N and MBN mg kg^{-1} in the unamended control soils were subtracted from the soil amended with each of the biosolids types and fertilisers, and then the values were expressed as a percentage of the total N added from each biosolids.

The total mineralised N which includes NO_3-N, NH_4-N and MBN of two biosolids and fertiliser, was calculated as a percentage of TN after subtracting the unamended control values. The total N added in inorganic form and mineral N sequestered in microorganism were estimated.

To estimate the mineralisable proportion of the organic N in the two biosolids the following formula was used (Smith and Durham, 2002):

Mineralisable N (% organic-N) = 100 x [Mineral $N_{measured}$ – (Mineral $N_{control}$ + Mineral N_{added})] / Organic-N_{added}

Where Mineral $N_{measured}$ and Mineral $N_{control}$ and Mineral N added includes the concentration of NO_3-N, NO_2-N, NH_4-N and MBN. Mineral N_{added} (mg N kg^{-1}) and organic-N added are from each of the biosolids types.

Total N sources were added from the two biosolids and two fertilisers are shown in Table 4.6. These amounts were based on the amount N required for growing perennial ryegrass (a crop used in the field experiments described in chapters 5 and 6).

Table 4.6 Initial N added from two biosolids and fertiliser types amended two soil types

Biosolids	Total N (mg N)	Mineral N (mg N)	Organic N (mg N)
Clay loam soil			
ANDB	1046	67	978
ADB	1046	131	915
Urea	512	-	512
NH_4Cl	512	512	-
Sandy loam soil			
ANDB	1082	70	1012
ADB	1082	135	946
Urea	530	-	530
NH_4Cl	530	530	-

4.3 Results and discussion

4.3.1 Physicochemical characterisation of soil and Biosolids

Soils

The soil samples taken from two different sites, MRWP with a clay loam soil and LA with sandy loam soil, had contrasting physicochemical characteristics which are presented in Table 4.7.

There was a considerable difference in organic matter and water holding capacity of the two soils; the clay loam soil had a high water holding capacity (62 %) compared to the sandy loam soil (39 %). Both soils were slightly acidic and had similar bulk densities. The clay loam soil was the most fertile with a high concentration of organic matter and sustained high moisture content associated with a greater biological activity as indicated by soil microbial biomass (C and N). The sandy loam soil from LA had a higher conductivity. The reasons for the higher salinity have been discussed in Chapter 3 (Section 3.2.2).

The total N content and inorganic N were greater in the clay loam soil than in the sandy loam soil, which is to be expected in a soil with a higher organic matter. The clay loam soil also had the higher inorganic N content. The total P, total S and heavy metals differed between the soils, but were within the normal range for agricultural use (Rigby et al., 2009).

Biosolids

The anaerobically digested dewatered biosolids used in this experiment from MRWP were categorized as C2 / T3 (contaminant grade 2 and treatment grade 3) (EPA, VIC ,2004), and had been stockpiled for 1 – 3 months. The aerobically digested biosolids produced at Barwon Water (BW) were classified as C2 / T2 and had been stockpiled for 3 years. As expected, the moisture content was low for ADB since the biosolids were exposed to environmental conditions such as wind, rainfall and heat during the summer. It is also expected that significant proportions of the NH_4-N and NO_3-N may have been lost through volatilization of NH_3-N or leaching of NO_3-N. All of the physicochemical properties for both biosolids were determined on dry weight basis using standard analytical procedures (Rayment and Lyons, 2011).

The EC value of ADB was significantly lower than ANDB which may be due to being stockpiled for three years.

The total N content was higher in the ANDB generated from MRWP mainly due to the higher organic N content. The fraction of inorganic N was higher in ADB than ANDB but the organic N of ANDB was significantly higher than ADB. The amount of inorganic and organic N in fresh biosolids will depend upon the treatment process. Once the biosolids are stockpiled, the available inorganic N will start leaching with rainfall events and the microbial population will start to break down the organic N to form mineral N, which in turn can be sequestered or leached. Consequently, the ratio of inorganic to organic N will depend upon the starting ratio, the time left in the stockpiles and the rate of microbial decomposition. The higher amount of PAN in ADB suggests that the treatment process for ANDB is more effective at stabilising N than aerobic digestion.

The concentrations of heavy metals were similar for both biosolids (Table 4.7).

Table 4.7 Selected physicochemical characteristics of soil and biosolids used in this study

Analytics	Clay loam soil	Sandyloam soil	ANDB	ADB
Moisture (%)	6.7 ± 0.9[c]	4.9 ± 0.1	28 ± 0.3	20 ± 0.8
pH	6.3 ± 0.05	5.6 ± 0.1	6.3 ± 0.02	5.2 ± 0.1
EC (µ S/ cm)	29.4 ± 11	57 ± 14	1220 ± 81	3.5 ± 0.2
OM (%)	7.3 ± 0.6	4.6 ± 1.0	66 ± 8	48 ± 6
Water holding capacity (%)	62.2 ± 0.2	39.1 ± 1.3	ND	ND
Bulk density (g/cm^3)	1.5 ± 0.1	1.4 ± 0.1	ND	ND
Total-N (%)	0.25	0.18	3.4	1.5
NH_4-N (µg/g)	4.3	4.4	155	1850
NO_3-N + NO_2-N (µg/g)	5.6	1.8	2050	93
Inorganic-N (µg/g)	9.9	6.2	2205	1943
Organic-N (µg/g)	2500	1800	31795	13557
PAN[a] (% Total N)	ND	ND	20.5[b]	34.4b
Total-C (%)	2.7	1.6	10.1	15.4
C:N	10.8	8.9	4.6	7.6
Total-P (µg/g)	6750	6990	13016	11726
Total-S (µg/g)	309	287	5424	6231
Olsen-P (µg/g)	2.6 ± 0.52	7.8 ± 0.5	48.0 ± 0.2	88.0 ± 0.9
Total Cu (mg/kg)	25 ± 3	8.4 ± 0.01	248 ± 4.5	202 ± 6
Total Zn (mg/kg)	38.6 ± 4.7	15 ± 0.01	570 ± 19	465 ± 11
Total Mn (mg/kg)	212 ± 8.7	146 ± 8	243 ± 5.8	369 ± 10
Total Ni (mg/kg)	35.7 ± 3.7	9.5 ± 0.4	45.4 ± 0.70	53 ± 1
Total Na (%)	0.19 ± 0.01	0.21 ± 0.01	0.26 ± 0.01	1.05 ± 0.01
Total K (%)	1.4 ± 0.04	0.73 ± 0.01	1.17 ± 0.01	1.99 ± 0.02
Total Ca (%)	0.43± 0.08	0.17 ± 0.01	2.14 ± 0.05	1.70 ± 0.01
Total Mg (%)	0.6 ± 0.06	0.21 ± 0.01	0.94 ± 0.01	1.28 ± 0.01
Total Al (%)	14.2 ± 0.5	6.03 ± 0.01	11.5 ± 0.01	11.4 ± 0.04
Total Fe (%)	0.05 ± 0.01	0.02 ± 0.01	7.4 ± 0.38	5.5 ± 0.04
MBC (mg/kg)	160 ± 13	79 ± 17	ND	ND
MBN (mg/kg)	47 ± 2	28 ± 1	ND	ND

[a] PAN in the biosolids was evaluated according to NLBAR ([NH_4-N] + [oxidised-N] + [MR × organic N])

[b] 15 % and 25% mineralisation of organic-N for anaerobically digested dewatered biosolids and aerobically digested biosolids, respectively, were used according the Victorian guideline for land application of biosolids.

ND not determined

[c] Mean ± standard deviation

All analytical results are expressed on air dry basis

4.3.2 Mineral N dynamics

Once organic N is converted to inorganic forms (NH_4-N and NO_3-N), it can be immobilised by transfer to the microbial biomass where it will not be immediately available to crops. It might also be responsible for lower N availability observed in less fertile soils (Smith et al., 1998a,HernÃ¡ndez et al., 2002,Rigby et al., 2009, Pu et al., 2012).

The C: N ratio of organic soil contributions affects the amount of mineralised N (Nicolardot et al., 2001,Vinten et al., 2002, Pansu and Thuriès, 2003). When the C: N ratio is high, as is generally the case in biosolids-amended soil, N is temporarily immobilised in microbial biomass as available C is assimilated. In studies conducted by Parker and Sommers (1983) it was shown that if an organic fertiliser source has a high C: N ratio, greater than 20:1, organic N may be immobilised by the microbial population, whereas when the C:N ratio is less than 20:1 the N content of the substrate is in excess of microbial cellular requirements and organic N tends to be mineralised.

The quantity of MBN is an important reservoir of N in biosolids-amended soils and is expected to change with soil type and biosolids. The stability of the added organic matter may be particularly important in modulating microbial and N dynamics in amended soils (Recous et al., 1990, Calbrix et al., 2007).

In the following sections, the production of NH_4-N, NO_3-N and MBN is displayed in mg of N kg^{-1} of soil for each amendment used (NH_4Cl, urea, ANDB and ADB). The change in MBC: MBN ratio has been calculated and is discussed in relation to the types of microorganisms likely to have been active. Finally, each N fraction is presented as a percentage of the total N applied. From these data, the time of maximum mineralisation can be ascertained, and an estimate made of the % mineralisable organic N contained in each of the biosolids applied.

The quantities of N mineralised for each day monitoring are shown in Table 4.8 and Table 4.9 for the clay loam and sandy loam soil respectively.

Table 4.8 Quantities of mineralisation rate of two biosolids and fertiliser types amended a clay loam soil

Time (days)	Treatment types	NO_3-N (mg kg^{-1})	NH_4-N (mg kg^{-1})	MBN (mg kg^{-1})	Mineral N recoveries (mg kg^{-1})*	Mineralised N (% TN)	Mineralised N (%TN) inorganic form	Mineralised N (%TN) (sequestered in microorganism)	Mineralised N (% organic-N)
1	Unamended	20 ± 3	16 ± 0.2	1.01 ± 0.05					
	ANDB	38 ± 4	35 ± 0.3	9.71 ± 0.2	46.2	13.82	11.21	2.60	7.69
	ADB	25 ± 0.5	43 ± 1.3	6.10 ± 0.4	36.4	10.55	9.07	1.47	-1.99
	Urea	25 ± 2	157 ± 0.2	2.5 ± 0.1	147	89.40	88.51	0.89	89.40
	NH_4Cl	22 ± 4	154 ± 1.5	2.4 ± 0.03	140	85.05	84.24	0.81	-
3	Unamended	31 ± 0.7	24± 0.6	5.8 ± 0.6					
	ANDB	75 ± 0.3	33 ± 1	13 ± 2	60.0	17.96	15.81	2.15	12.07
	ADB	50 ± 0.6	50 ± 2	10 ± 0.3	48.7	14.09	12.90	1.18	10.29
	Urea	54 ± 2	123 ± 3.4	10 ± 2	126	76.60	74.03	2.57	91.12
	NH_4Cl	43 ± 2	135 ± 6	3.4 ± 2.5	120	73.07	74.49	-1.42	-
8	Unamended	28 ± 0.8	26 ± 0.5	8.7 ± 0.1					
	ANDB	119 ± 0.7	33 ± 3	16 ± 0.3	104	31.34	29.14	2.21	26.25
	ADB	92 ± 3	41 ± 1.1	16.5 ± 1	85.5	24.72	22.47	2.25	23.57
	Urea	104 ± 0.6	120 ± 7.4	10 ± 0.2	171	103.42	102.66	0.76	119
	NH_4Cl	72± 0.3	101 ± 13	9.5 ± 0.3	120	72.52	72.05	0.46	-
14	Unamended	17 ± 2	25± 4.5	29 ± 3					
	ANDB	199 ± 2	21 ± 5	51 ± 0.7	199	59.74	53.28	6.46	56.31
	ADB	107 ± 0.7	21 ± 65	50 ± 0.2	107	30.95	24.98	5.97	33.49
	Urea	256 ± 1	120 ± 3	41 ± 1.3	346	209.1	202.0	7.19	229
	NH_4Cl	88 ± 0.3	72 ± 20	22 ± 0.2	110	66.95	71.19	-4.24	-

Cont. Table

Time (days)	Treatment types	NO_3-N (mg kg^{-1})	NH_4-N (mg kg^{-1})	MBN (mg kg^{-1})	Mineral N recoveries (mg kg^{-1})*	Mineralised N (% TN)	Mineralised N (%TN) inorganic form	Mineralised N (%TN) (sequestered in microorganism)	Mineralised N (% organic-N)
28	Unamended	26 ± 1	1 ± 0.1	31 ± 0.5					
	ANDB	229 ± 1.2	0.9 ± 0.1	80 ± 1.3	252	75.39	60.66	14.74	72.89
	ADB	147 ± 0.8	0.8 ± 1	72 ± 2	161	46.64	34.92	11.72	47.56
	Urea	219 ± 1	113 ± 18	59 ± 2.8	333	201.67	184.92	16.75	220
	NH_4Cl	82 ± 0.53	78 ± 13	33 ± 0.7	133	80.94	80.16	0.77	-
42	Unamended	33 ± 0.8	1 ± 0.8	38 ± 0.3					
	ANDB	277 ± 2	1 ± 0.04	73 ± 4	279	83.51	72.98	10.53	81.49
	ADB	216 ± 1.2	0.8 ± 0.04	85 ± 4.4	230	66.57	52.90	13.67	75.41
	Urea	283 ± 25	93 ± 12	68 ± 2	373	225.54	207.13	18.41	282
	NH_4Cl	63 ± 2	32 ± 4.3	60 ± 6.7	82	49.75	36.72	13.03	-
56	Unamended	40 ± 5	4. 4 ± 4	14.3 ± 0.4					
	ANDB	364 ± 21	0.5 ± 0.16	49.4 ± 0.7	355	106.29	95.79	10.51	105.60
	ADB	314 ± 9	0.7 ± 0.5	67.7 ± 1.4	324	93.73	78.29	15.44	102.80
	Urea	122 ± 5	62 ± 1	65.2 ± 2.3	191	115.52	84.74	30.78	134
	NH_4Cl	52 ± 1	21 ± 5.5	13.4 ± 1	27.4	16.56	17.05	-0.50	-
70	Unamended	22 ± 0.9	4.7 ± 4	5.61 ± 0.8					
	ANDB	183 ± 0.2	5 ± 1	16.7 ± 1.9	172	51.60	48.27	3.33	47.69
	ADB	178 ± 2	2.2 ± 1.2	28.8 ± 1.9	176	51.05	44.34	6.71	43.99
	Urea	79 ± 0.2	46± 8	50.1 ± 1	142	86.22	59.33	26.89	83.66
	NH_4Cl	45 ± 0.6	15 ± 13	3.08 ± 1	29.8	18.01	19.54	-1.53	-

Cont. Table

Time (days)	Treatment types	NO_3-N (mg kg^{-1})	NH_4-N (mg kg^{-1})	MBN (mg kg^{-1})	Mineral N recoveries (mg kg^{-1})*	Mineralised N (% TN)	Mineralised N (%TN) inorganic form	Mineralised N (%TN) (sequestered in microorganism)	Mineralised N (% organic-N)
84	Unamended	24 ± 2	2 ± 1	2.44 ± 0.2					
	ANDB	149 ± 1	6 ± 0.1	16.0 ± 0.2	139	41.66	37.60	4.05	37.17
	ADB	119 ± 2	5 ± 4	11.4 ± 0.6	103	29.94	27.32	2.61	18.93
	Urea	48 ± 0.1	28 ± 21	13.5 ± 0.6	57.2	34.58	27.86	6.73	31.41
	NH_4Cl	29 ± 0.5	11 ± 8	6.89 ± 0.08	15.1	9.11	6.42	2.69	-
91	Unamended	27 ± 2	2.4 ± 0.11	0.93 ± 0.3					
	ANDB	56 ± 4	4.5 ± 0.3	0.85 ± 0.2	9.75	9.75	9.77	-0.02	3.38
	ADB	56 ± 2	3 ± 0.6	4.22 ± 0.2	10.30	9.96	9.00	0.95	-5.56
	Urea	36 ± 0.4	16 ± 2.7	1.83 ± 0.06	15.16	15.16	14.61	0.55	10.02
	NH_4Cl	23 ± 0.3	9 ± 8	3.99 ± 0.1	4.58	4.58	2.72	1.85	-

*Mineral N recoveries (NO_3-N, NH_4-N and MBN) after subtracted from the unamended control soil

Table 4.9 Quantities of mineralisation rate of two biosolids and fertiliser types amended a sandy loam soil

Time (days)	Treatment types	NO_3-N (mg kg^{-1})	NH_4-N (mg kg^{-1})	MBN (mg kg^{-1})	Mineral N recoveries (mg kg^{-1})*	Mineralised N (% TN)	Mineralised N (%TN) inorganic form	Mineralised N (%TN) (sequestered in microorganism)	Mineralised N (% organic-N)
1	Unamended	2 ± 0.6	19 ± 3	1.6 ± 0.2					
	ANDB	15 ± 2	27 ± 0.4	5.5± 0.1	25.5	7.63	6.48	1.15	0.87
	ADB	4 ± 1.3	57 ± 0.1	5.4 ± 0.5	43.8	12.67	11.58	1.09	0.00
	Urea	2 ± 1.2	158 ± 1.7	1.4 ± 0.05	139	81.16	81.32	-0.16	81.16
	NH_4Cl	0.5 ± 0.1	151 ± 2.6	3.9 ± 0.3	133	77.64	76.37	1.27	-
3	Unamended	15 ± 0.8	23 ± 1.4	2.5± 0.4					
	ANDB	31 ± 5	53 ± 1.3	6.8 ± 3	50.1	15.00	13.73	1.27	8.42
	ADB	47 ± 1	42 ± 1.1	5.9± 1.3	53.9	15.61	14.63	0.99	3.33
	Urea	18 ± 1	177 ± 4.2	4.7 ± 1.4	159	93.11	91.83	1.28	93.11
	NH_4Cl	11 ± 0.5	135 ± 2.6	1.9 ± 0.8	107	62.78	63.12	-0.34	-
8	Unamended	24 ± 5	26 ± 0.2	8.1 ± 0.3					
	ANDB	81 ± 0.9	49 ± 19	9.4 ± 0.2	81.9	24.49	23.97	0.52	18.1
	ADB	105 ± 0.7	48 ± 6.2	9.6 ± 0.2	105	30.41	29.97	0.44	20.1
	Urea	53 ± 0.2	184 ± 14	9.7 ± 0.2	190	110.99	109.89	1.10	110.9
	NH_4Cl	29 ± 0.2	136 ± 24	6.7 ± 0.2	114	66.69	67.48	-0.79	-
14	Unamended	20 ± 0.2	27 ± 3.2	23 ± 0.3					
	ANDB	88 ± 0.5	45 ± 1.5	30 ± 0.5	92.5	27.68	25.82	1.87	21.4
	ADB	182 ± 0.6	29 ± 6.7	20 ± 1.7	162	46.87	47.73	-0.85	38.7
	Urea	110 ± 1.3	81 ± 3.3	18 ± 0.5	140	81.72	84.76	-3.05	81.72
	NH_4Cl	33 ± 0.2	107 ± 25	23 ± 1.2	93	54.22	54.33	-0.11	-

Cont. Table

Time (days)	Treatment types	NO_3-N (mg kg^{-1})	NH_4-N (mg kg^{-1})	MBN (mg kg^{-1})	Mineral N recoveries (mg kg^{-1})*	Mineralised N (% TN)	Mineralised N (%TN) inorganic form	Mineralised N (%TN) (sequestered in microorganism)	Mineralised N (% organic-N)
28	Unamended	21 ± 0.4	1.5 ± 1.1	27 ± 0.7					
	ANDB	161 ± 1.7	5 ± 3.45	54 ± 0.1	170	51.00	42.89	8.10	45.3
	ADB	142 ± 5	7.7 ± 4.7	35 ± 0.1	134	38.95	36.90	2.06	29.7
	Urea	69 ± 1.2	43 ± 37.7	23 ± 0.7	85	49.60	52.16	-2.56	49.60
	NH_4Cl	28 ± 0.2	49 ± 10	32 ± 0.6	59	34.74	31.98	2.76	-
42	Unamended	26 ± 0.8	1.4 ± 0.25	32 ± 0.3					
	ANDB	170 ± 0.9	4.9 ± 1.01	29 ± 1.3	145	43.52	44.37	-0.85	37.6
	ADB	163 ± 2	7.7 ± 0.14	43 ± 0.6	154	44.74	41.58	3.15	36.3
	Urea	112 ± 3	43 ± 4	15 ± 0.7	112	65.28	75.07	-9.80	65.28
	NH_4Cl	58 ± 1	27 ± 6.7	14 ± 0.5	40	23.48	33.84	-10.36	-
56	Unamended	29 ± 0.5	0.6 ± 0.6	12 ± 0.1					
	ANDB	296 ± 1	2.5 ± 2.4	22 ± 2.6	279	83.62	80.47	3.15	78.68
	ADB	167 ± 0.6	1.07 ± 2.6	41 ± 2.7	167	48.56	40.00	8.55	40.60
	Urea	69 ± 0.9	23.2 ± 9	26 ± 0.7	76.9	44.88	36.32	8.56	44.88
	NH_4Cl	44 ± 0.1	19.5 ± 5	9.5 ± 0.6	31.1	18.18	19.53	-1.36	-
70	Unamended	13 ± 0.6	4.43 ± 0.2	1.9 ± 0.7					
	ANDB	191 ± 1	6.7 ± 2.3	21 ± 0.1	200	59.81	53.85	5.96	54.3
	ADB	144 ± 0.4	6.1 ± 1.6	24 ± 0.6	155	44.92	38.55	6.37	36.5
	Urea	41 ± 1	22 ± 4	24 ± 0.1	68	39.73	26.38	13.35	39.73
	NH_4Cl	47 ± 0.2	25 ± 1.6	5.4 ± 0.3	57.8	33.80	31.79	2.01	-

Cont. Table

Time (days)	Treatment types	NO_3-N (mg kg^{-1})	NH_4-N (mg kg^{-1})	MBN (mg kg^{-1})	Mineral N recoveries (mg kg^{-1})*	Mineralised N (% TN)	Mineralised N (%TN) inorganic form	Mineralised N (%TN) (sequestered in microorganism)	Mineralised N (% organic-N)
84	Unamended	11 ± 0.4	3.2 ± 1	1.8 ± 0.1					
	ANDB	121 ± 0.7	4.4 ± 0.4	10 ± 0.1	119	35.56	33.12	2.45	29.5
	ADB	94 ± 0.2	7.3 ± 0.2	1.8 ± 0.3	87	25.22	25.21	0.01	14.2
	Urea	34 ± 0.2	14 ± 0.6	11 ± 1.7	43	25.18	19.69	5.49	25.18
	NH_4Cl	23 ± 0.2	18.2 ± 0.5	5.22 ± 0.1	30	17.39	15.37	2.02	-
91	Unamended	9.8 ± 0.2	3.3 ± 0.8	2.7 ± 0.1					
	ANDB	38 ± 0.1	3.9 ± 1.1	7.2 ± 0.3	34	10.09	8.74	1.35	3.4
	ADB	32 ± 9	5.9 ± 1	0.6 ± 0.1	22	6.52	7.15	-0.63	-7.0
	Urea	33 ± 0.2	8.0 ± 2.1	1.8 ± 0.1	27	15.55	16.08	-0.53	15.55
	NH_4Cl	16 ± 0.2	10.5 ± 1.1	0.8 ± 0.05	12	6.89	8.01	-1.11	-

*Mineral N recoveries (NO_3-N, NH_4-N and MBN) after subtracted from the unamended control soil

4.3.2.1 Mineral N dynamics of NH_4Cl in the clay loam soil

The rate of ammonification (NH_4-N) and nitrification (NO_3-N) processes of organic-N in NH_4Cl-applied to the clay loam soil soils is presented in Figure 4.4. The figures show results from triplicate samples, which are in very close agreement.

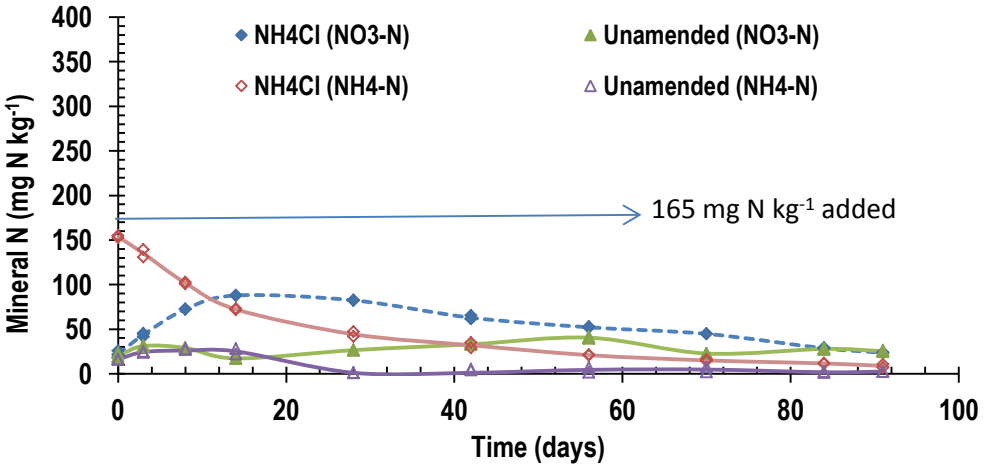

Figure 4.4. The effect of applying NH_4Cl on NO_3-N and NH_4-N dynamics on the clay loam soil (n=3) (the connecting lines refers to the means of triplicate measurements plotted against time)

Production of mineral N (NH_4-N and NO_3-N) from NH_4Cl

The concentration of NH_4-N obtained from the clay loam soil amended with NH_4Cl initially increased and reached a maximum value of 152 mg N kg^{-1} on day 1 (Figure 4.4). The amount of NH_4-N at the beginning of trial was six times greater than the amount of NH_4-N in the unamended control soil. However, NH_4-N levels gradually decreased (Figure 4.4).

As NH_4-N decreased, the concentration of NO_3-N increased reaching a maximum on day 14 (87 mg N kg^{-1}), after which it gradually decreased. As expected, it was observed that the amount of NO_3-N produced from the fertiliser was greater than the concentration of NO_3-N measured in the unamended control soil over the same period. Most of the N was measured as NH_4-N, and as NH_4-N decreased as expected (Smith and Tibbett, 2004, Hseu and Huang, 2005, Pu et al., 2008, Rigby et al., 2009, Lu et al., 2012), there was a corresponding increase in NO_3-N levels.

4.3.2.2 Microbial biomass N and C

The concentrations of Microbial biomass N and C in NH_4Cl-treated the clay loam soil are shown in Figure 4.5.

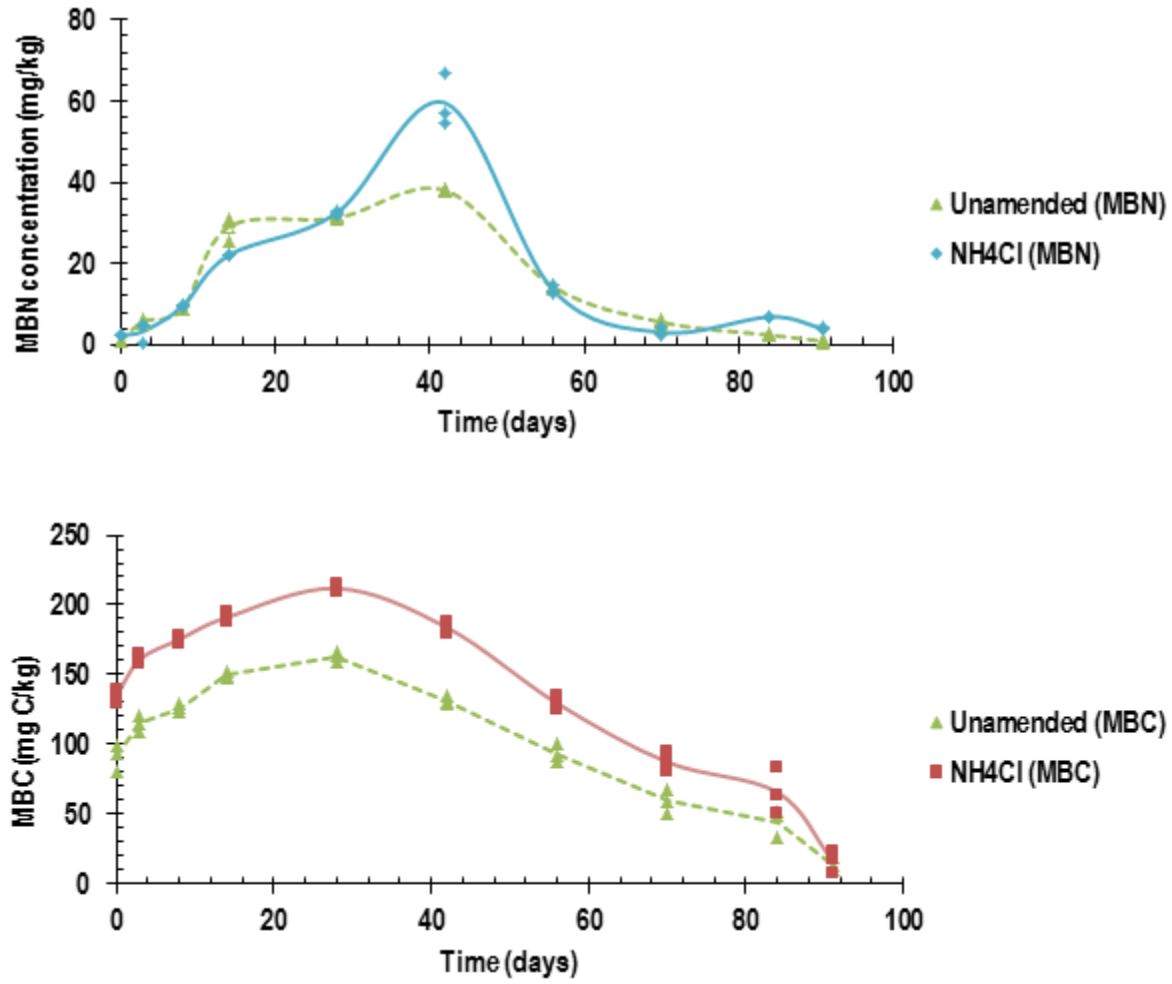

Figure 4.5. The influence of applying NH_4Cl on MBN and MBC dynamics on clay loam soil (n=3) (the connecting lines refers to the means of triplicate measurements plotted against time)

The microbial biomass N (MBN) concentration in NH_4Cl-amended clay loam soil was between 2 – 60 mg N kg^{-1} from the initial concentration to day 42. This consistent with work done by Zaman et al., (2002) who observed that an increase in MBN concentration following soil amendment with NH_4Cl. The quantity of N immobilised from NH_4Cl treated clay loam soil was 58 % higher than the content of MBN obtained in the unamended control soil (Figure 4.5). The increase of MBN is corresponds with increasing nitrification as shown in Figure 4.4.

The concentration of MBC increased to a maximum of 214 mg C kg^{-1} on day 28 (Figure 4.5). At its maximum, the quantity of MBC was 26 % greater than the amount observed in the unamended control soil. The increase in MBC in the NH_4Cl would be related to the available C fraction in the soil background which stimulated microbial activity during this period. The levels of MBC in the NH_4Cl-amended and unamended control then decreased. It may be due to the exhaustion of this available C substrate. Similar observations of MBC reported by Frampton et al.,(1999) after 16 days following the NH_4Cl-applied to soil.

Microbial biomass ratio

Microbial biomass C to N ratio in NH_4Cl-amended clay loam soil over 91 days is shown in

Figure **4.6**. In the unamended clay loam soil, the ratio was highest at day 1. It deceased until day 42 and then increased again. The results of increasing of C: N ratio of the biomass towards the end of the experiment may indicate that fungi gradually developed during this period. This is increase is consistent with work done by Kao et al., (2006) who showed that a C:N biomass ratio of about 8 is consistent with terrestrial bacteria while a higher ratio of 13 is consistent with the growth of fungi. The ratios determined in the current work were determined using extraction efficiency data from literature which may not have been correct for this work. So while the actual ratios may not completely correspond to the work of Kao et al., (2006) the change in magnitude does.

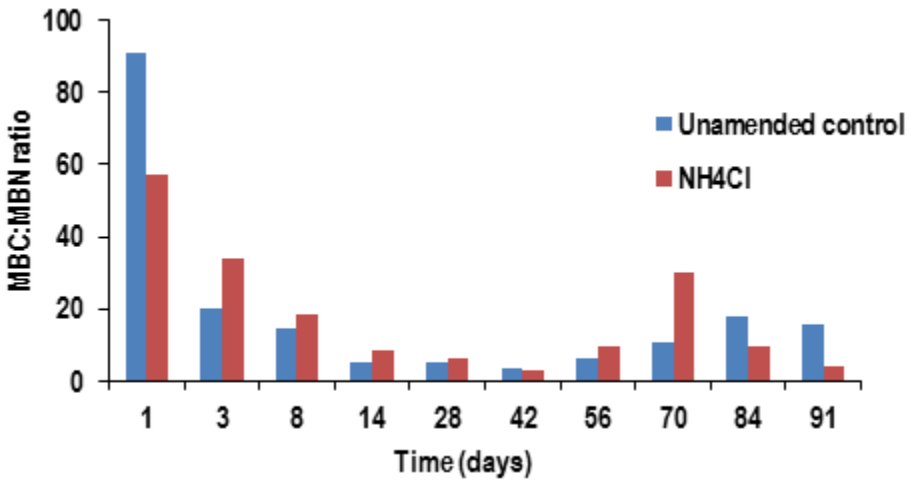

Figure 4.6. The ratio of microbial biomass C to N (mean, n = 3) in the NH_4Cl treated clay loam soil

4.3.2.3 Mineral N dynamics of NH_4Cl in the sandy loam soil

The rate of ammonification (NH_4-N) and nitrification (NO_3-N) processes of organic-N in NH_4Cl-applied to the sandy loam soil soils is presented in Figure 4.7.

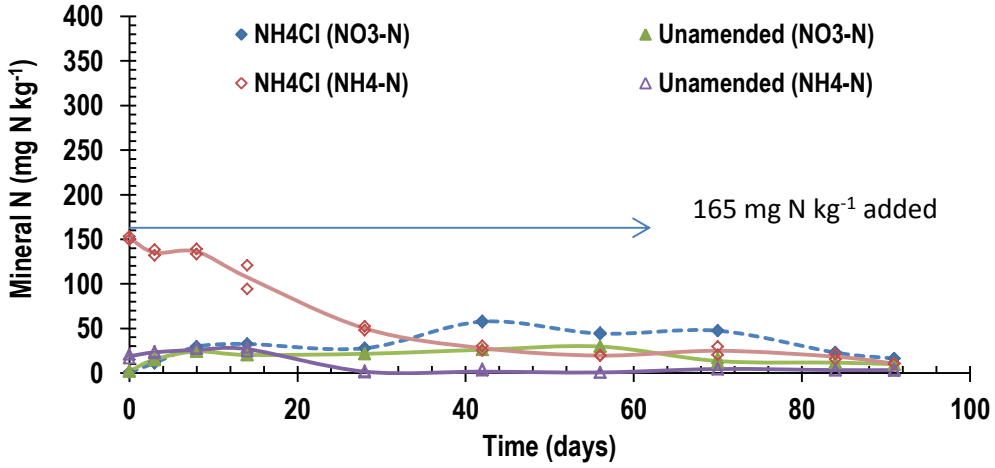

Figure 4.7. The effect of applying NH_4Cl on NO_3-N and NH_4-N dynamics on the sandy loam soil (n=3) (the connecting lines refers to the means of triplicate measurements plotted against time)

Production of mineral N (NH_4-N and NO_3-N) from NH_4Cl

The concentration of NH_4-N observed from NH_4Cl-amended sandy loam soil reached a maximum (152 mg N kg^{-1}) within 1 day after incubation period and then decreased

over the remaining 91 days (Figure 4.7). This is consistent with amount observed from NH_4Cl treated the clay loam soil.

The level of NO_3-N in NH_4Cl-amended sandy loam soil was 59 mg N kg^{-1} on day 42. As with the clay loam soil, the concentration of NO_3-N in NH_4Cl-treated soil was higher than the corresponding level of the NO_3-N in the unamended control soil. The delay of nitrification process in this soil may be due to less organic-N than the amount of organic-N in the clay loam soil.

4.3.2.4 Microbial biomass N and C

The concentration of MBN and MBC in NH_4Cl-amended the sandy loam soil over 91 days is presented in Figure 4.8.

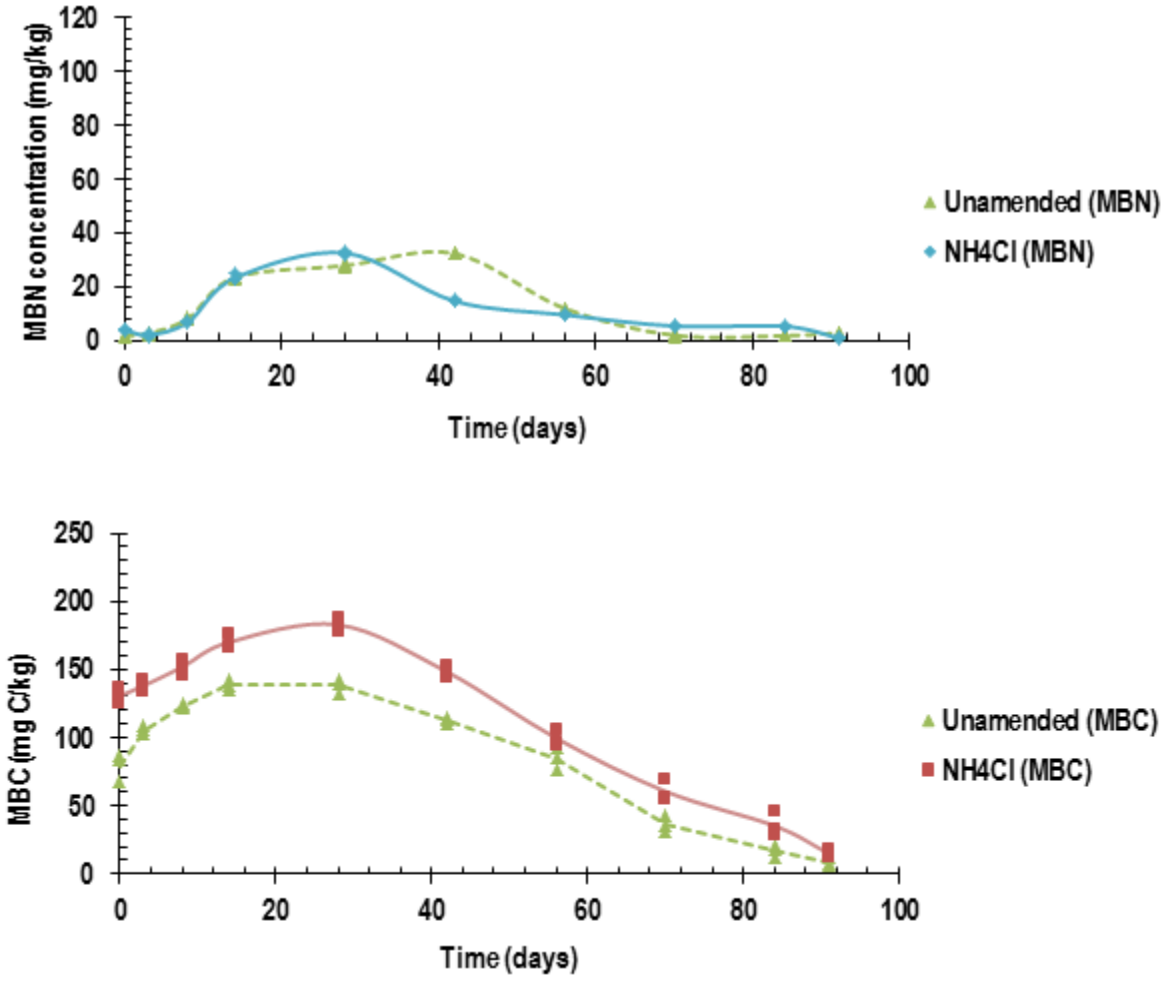

Figure 4.8. The influence of applying NH_4Cl on MBN and MBC dynamics on sandy loam soil (n=3) (the connecting lines refers to the means of triplicate measurements plotted against time)

The MBN concentration in NH_4Cl-amended sandy loam soil was between 2 – 33 mg N kg^{-1} from day 1 to day 28. The quantity of N immobilised from NH_4Cl-treated sandy loam soil was 18 % higher than the content of MBN obtained in the unamended control soil (Figure 4.8). The MBN content in the sandy loam soil was lower than the clay loam soil.

The concentration of MBC increased to a maximum of 178 mg C kg^{-1} on day 28. The quantity of MBC was 34 % greater than the amount observed in the unamended control soil. The levels of MBC in the NH_4Cl-amended and unamended control then decreased. By day 28, the microbial biomass C reached the maximum concentrations in both soils but it was higher in the clay loam soil which may be due to more organic matter and nutrients than the sandy loam soil.

Microbial biomass ratio

Microbial biomass C to N ratio in NH_4Cl-treated sandy loam soil over 91 days is shown in Figure 4.9. The ratio of MBC: MBN was high following the application rate of NH_4Cl and then decreased until day 28. After day 28, the ratio increased again, possibly due to development of fungi (KAO et al., 2006) as in the clay loam soil.

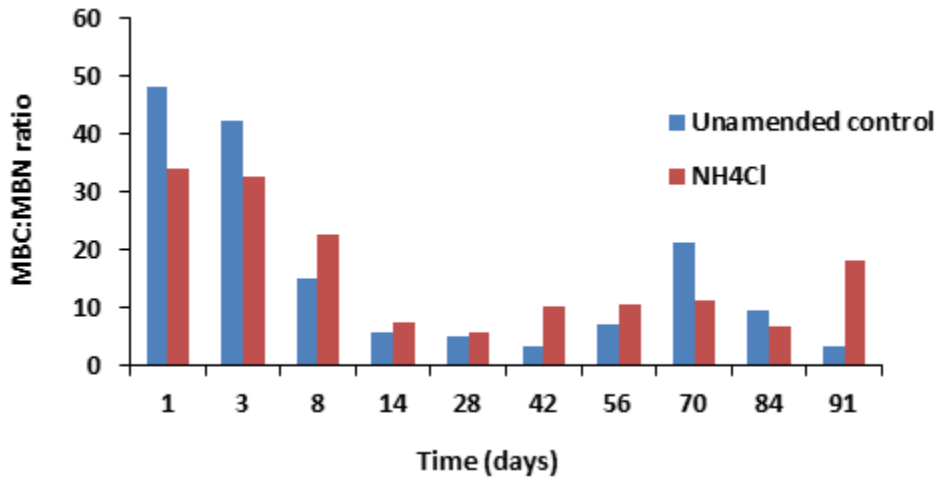

Figure 4.9. The ratio of microbial biomass C to N (mean, n = 3) in the NH_4Cl-treated sandy loam soil

Comparison between two soil types amended with NH_4Cl

The amount of total N added from NH_4Cl in two soil types was approximately 85 % as shown in Figure 4.10, which is consistent with the application of NH_4Cl to three different soil types under similar laboratory incubation experiment done by Rigby and Smith (2013). The total mineral N (%TN added) observed in their study was 87 % on day 0.

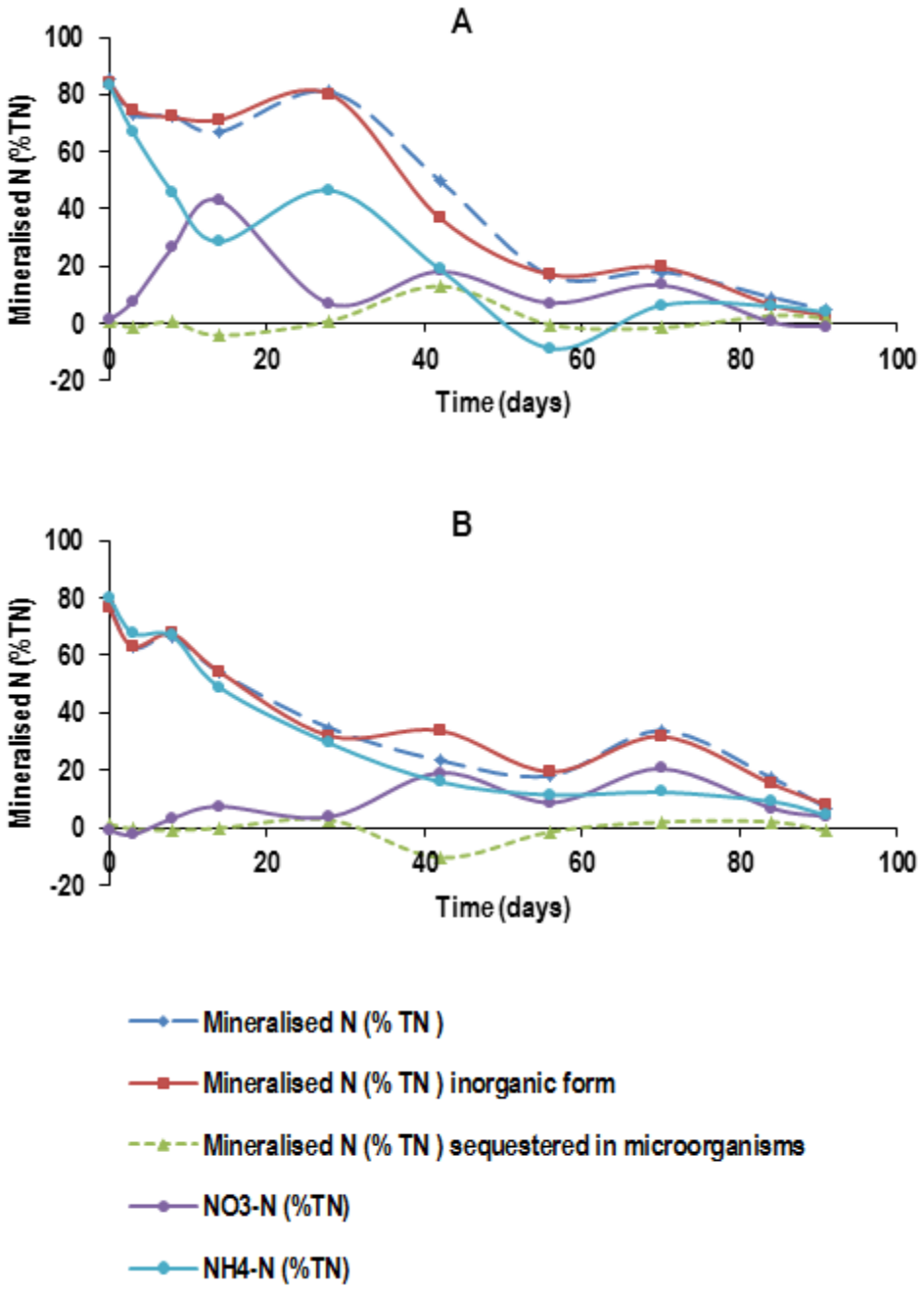

Figure 4.10. Mineral N (%TN added) from NH_4Cl-applied to the clay loam (A) and sandy loam soil (B)

The total N added from NH_4Cl was 165 mg N kg^{-1} and the concentrations of NH_4-N, NO_3-N and MBN expressed as a percentage of total N are shown in Table 4.10. The amount of organic-N mineralised was calculated using the formula of Smith and Durham (2002) as described in section 4.2.5.

Table 4.10 shows the maximum values mineral N (NH_4-N and NO_3-N) and MBN as % TN added after subtraction of concentrations in the unamended control soil.

Table 4.10 Mineral N from NH_4Cl-amended two soil types under laboratory incubation trial

NH_4Cl	Day of maximum mineralisation N	% TNadded NH_4-N	% TNadded NO_3-N	% TNadded MBN	Mineralised N (% TN)
Clay loam soil	1	83 ± 1	1 ± 1	0.8 ± 0.1	85 ± 2
Sandy loam soil	1	80 ± 2	-1 ± 1	1.3 ± 0.6	78 ± 3

Following the application of NH_4Cl to the clay loam and sandy loam soil, the amount of NH_4-N observed similar in both soil at the beginning of the experiment. It can be seen that there was no effect of soil types on the N mineralised as total N added from this amendment on day 1.

4.3.2.5 Mineral N dynamics of urea in the clay loam soil

The rate of ammonification (NH_4-N) and nitrification (NO_3-N) processes of organic-N in urea -applied to the clay loam soil soils is presented in Figure 4.11.

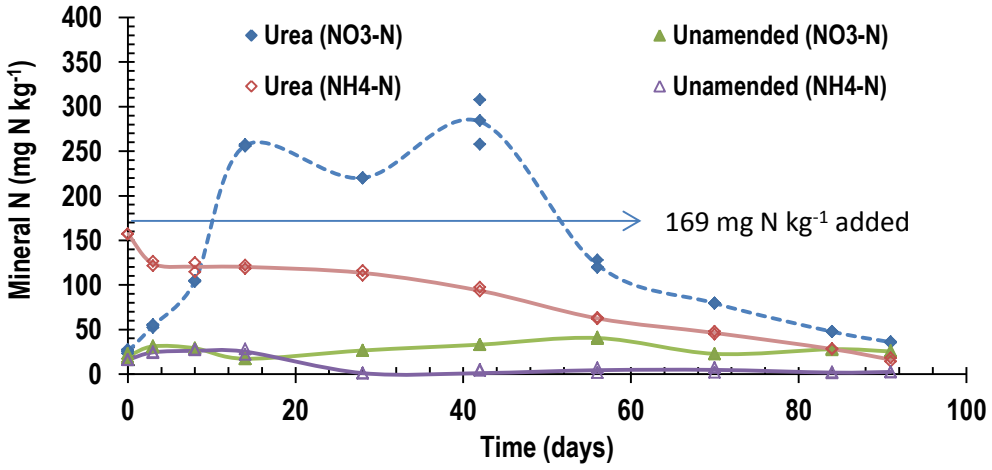

Figure 4.11. The effect of applying urea on NO_3-N and NH_4-N dynamics on the clay loam soil (n=3), (the connecting lines show the mean of triplicate measurements plotted against times)

Production of mineral N (NH_4-N and NO_3-N) from urea

The concentration of NH_4-N observed in the clay loam soil amended with urea, decreased gradually from day 1, over 91 days (157 mg N kg^{-1} to 2 mg N kg^{-1}). In contrast, the NH_4-N observed in the unamended control soil initially increased to 28 mg N kg^{-1} by day 14, but dropped to below the detection level by day 28 (Figure 4.11). The change in the concentration of NH_4-N corresponded to an increase in the concentration of NO_3-N over the same period.

The concentration of NO_3-N increased noticeably between days 1 – 14. The concentration of NO_3-N in urea-amended clay loam soil increased to 256 mg N kg^{-1} compared to 28 mg N kg^{-1} measured in the unamended clay loam control soil on day 14. The NO_3-N produced with urea was 19 mg N kg^{-1} day^{-1}. The rapid nitrification during this period is presumably due to a result of soil microbial activity in the fertile clay loam soil. However, by day 42 the levels of NO_3-N in urea-treated clay loam soil reached a maximum value at 283 mg N kg^{-1}. Hence, the accumulation of NO_3-N on day 42 was 6 mg N kg^{-1} day^{-1}, the amount of NO_3-N produced from urea was approximately seven times higher than the amount found in the unamended control soil. The concentration of NO_3-N in the urea-amended soil decreased after day 42, whereas the levels of NO_3-N in the unamended soil continued to increase until day 56 reaching a maximum of 39 mg N kg^{-1} (Figure 4.11). There was an error on the NO_3-N produced between days 14 - 42, which might be the amount of N added more than the amount observed.

4.3.2.6 Microbial biomass N and C

The concentration of MBN and MBC in urea-amended the clay loam soil over 91 days is presented in Figure 4.12.

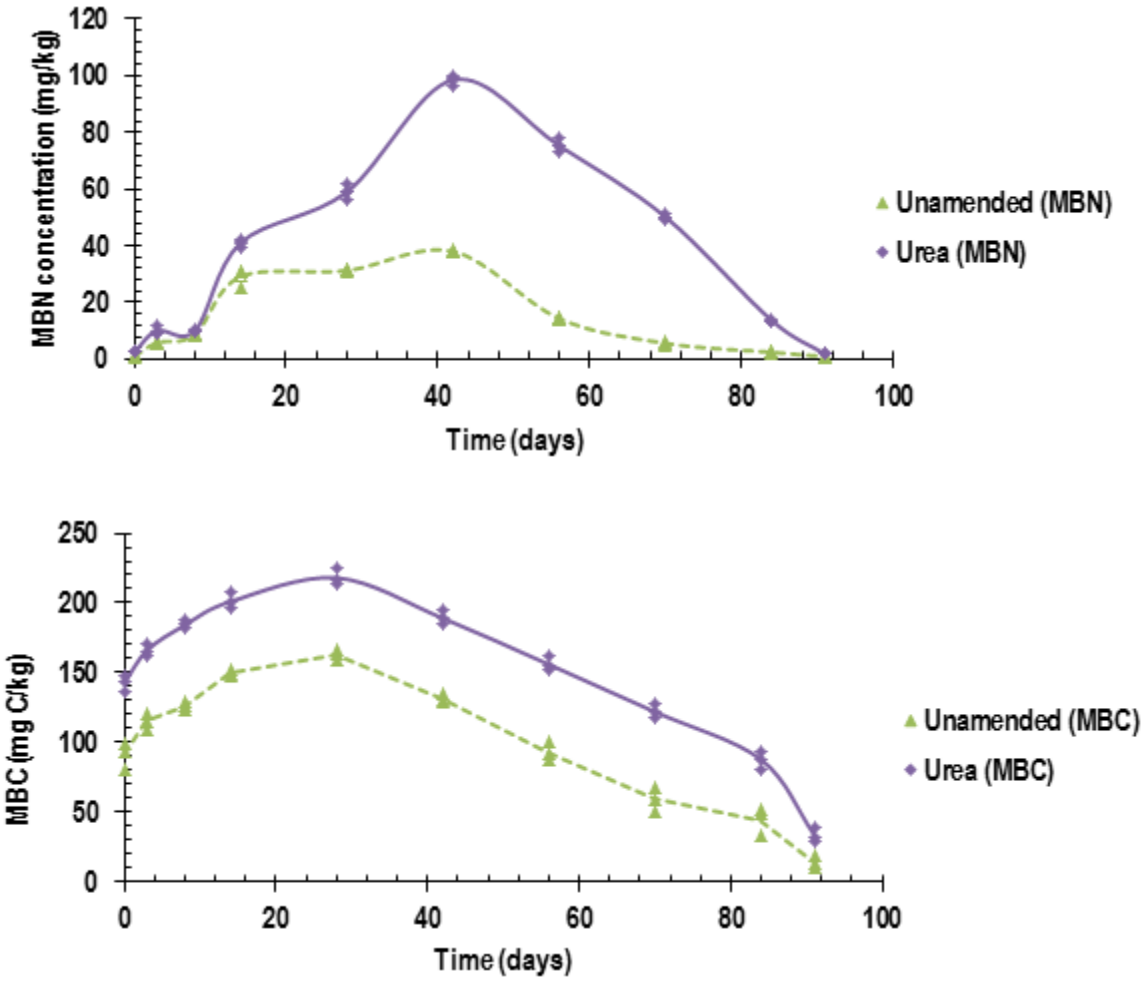

Figure 4.12. The influence of applying urea on MBN and MBC dynamics on clay loam soil (n=3) (the connecting lines refers to the means of triplicate measurements plotted against times)

In the clay loam soil amended with urea, the MBN concentration increased and reached a maximum value of 98 mg N kg^{-1} on day 42 compared to 38 mg N kg^{-1} of MBN content measured in the unamended control soil at the same period. It may be presumed that, in that period, the amount of N immobilised be soil microbial population was approximately one and half times greater than the mass of MBN content detected in the unamended control soil. Nevertheless, by day 42 onwards the levels of MBN decreased until the end of the incubation period (Figure 4.12).

The level of MBC in urea-treated the clay loam soil reached a maximum at 224 mg C kg^{-1} on day 28. Others have observed the same increase in MBC following the application of urea over a short-term incubation period (Stark et al., 2007). However, the levels of MBC in urea and unamended control decreased after day 28. The

quantity of MBC was 35 % higher than the amount observed in the unamended control soil.

Microbial biomass ratio

Microbial biomass C to N ratio in urea-amended clay loam soil over 91 days is shown in Figure 4.13. The ratio of MBC: MBN in urea was higher at the beginning and then decrease until day 56. However, the MBC: MBN ratio of biomass increased from day 70 to day 91, it behaved the same way as the NH_4Cl treated soils.

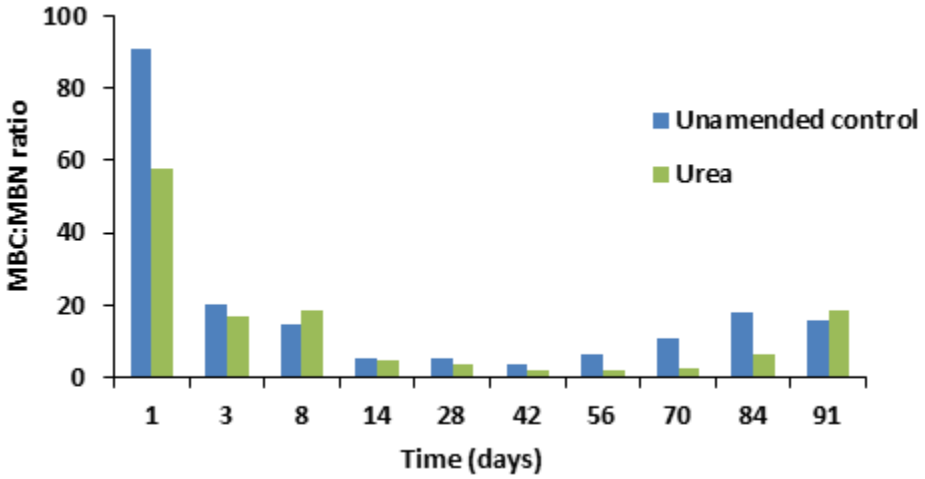

Figure 4.13. The ratio of microbial biomass C to N (mean, n = 3) in the NH_4Cl amended clay loam soil

4.3.2.7 Mineral N dynamics of urea in the sandy loam soil

The rate of ammonification (NH_4-N) and nitrification (NO_3-N) processes of organic N in urea-applied to the sandy loam soil soils are shown in Figure 4.14.

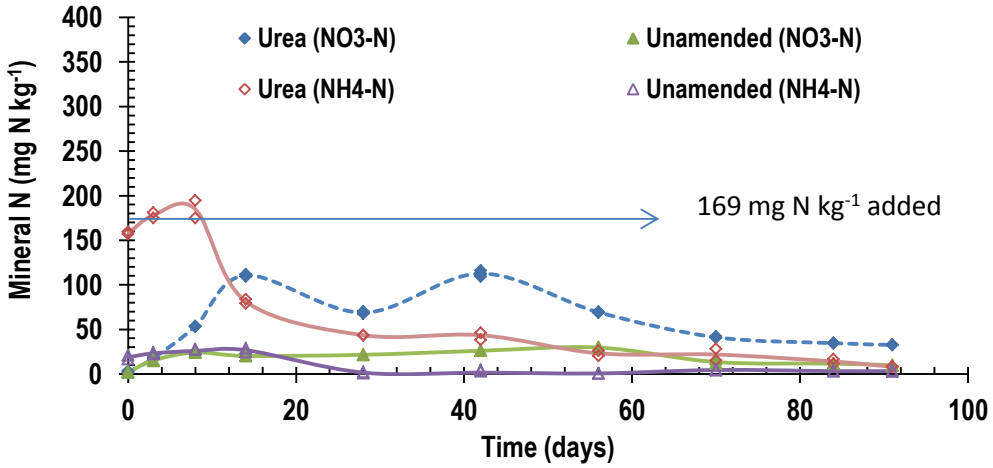

Figure 4.14. The effect of applying urea on NO_3-N and NH_4-N dynamics on the sandy loam soil (n=3) (the connecting lines refers to the means of triplicate measurements plotted against times)

Production of mineral N (NH_4-N and NO_3-N) from urea

The concentration of NH_4-N observed from urea-amended sandy loam soil reached a maximum at 194 mg N kg^{-1} on day 8 and then decreased.

In the sandy loam soil amended with urea, the levels of NO_3-N increased to 116 mg N kg^{-1} which is equivalent to 8 mg N kg^{-1} day^{-1} on day 42. Thus, the quantity of NO_3-N produced from urea-amended sandy loam soil was three times higher than the production observed in the unamended control soil. After day 42, the concentration of NO_3-N released in the urea-treated soil decreased over 91 days.

4.3.2.8 Microbial biomass N and C

The concentration of MBN and MBC in urea-amended the sandy loam soil over 91 days is presented in Figure 4.15.

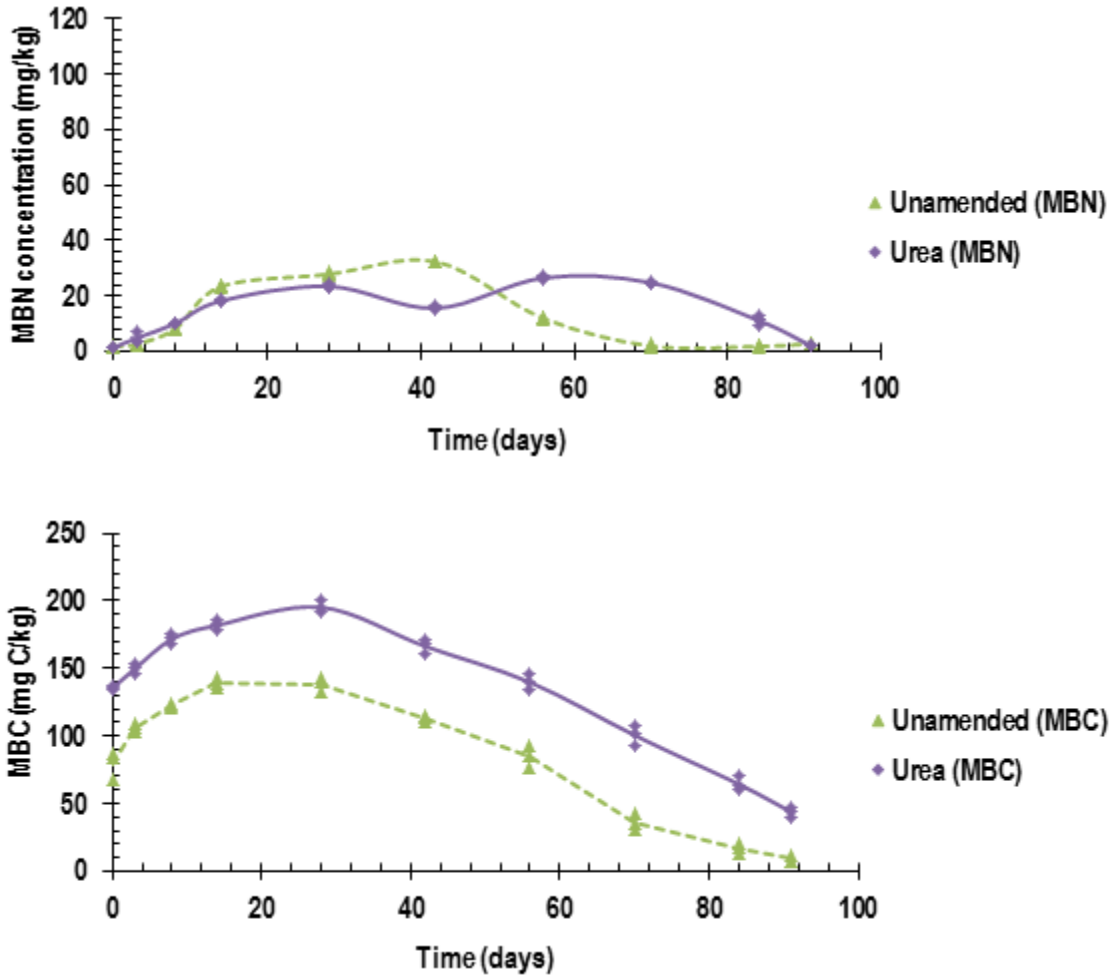

Figure 4.15. The influence of applying urea on MBN and MBC dynamics on sandy loam soil (n=3) (the connecting lines refers to the means of triplicate measurements plotted against times)

The concentration of MBN in urea-amended sandy loam soil increased from the initial concentration of MBN to day 28. Meanwhile, by day 56 the level of MBN in sandy loam soil amended with urea reached a maximum value at 26 mg N kg^{-1}. The MBN concentrations observed in the sandy loam soil was lower than the clay loam soil receiving urea as shown in Figure 4.12.

The greatest MBC concentration observed in urea-amended sandy loam soil was 195 mg C kg^{-1} and on day 28 and then decreased. A similar pattern of MBC concentration in NH_4Cl-amended sandy loam soil was observed.

Microbial biomass ratio

Microbial biomass C to N ratio in urea-amended the sandy loam soil over 91 days is shown in Figure 4.16. The ratio of MBC: MBN was higher within the first 10 days incubation period and then decrease to lower values by day 28. However, the biomass ratio increased between days 42 – 91.

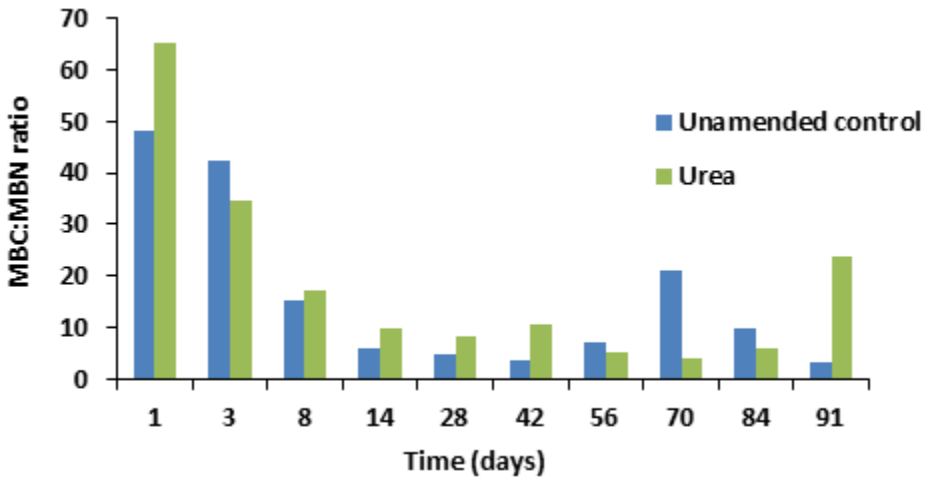

Figure 4.16. The ratio of microbial biomass C to N (mean, n = 3) in the urea treated sandy loam soil

Comparison between two soil types amended with Urea

Unexpectedly, the N availability from urea-amended the clay loam soil was more than total N added (Figure 4.17). It looks as though an error was made, but efforts to identify any error were not successful. Nevertheless, the trend in production of NH_4-N and NO_3-N can be clearly seen. For more exploration of this error see Appendix B.

Figure 4.17 shows that the total mineral N (% TN added), total of inorganic from (% TN added) mineralised and total microbial biomass N (% TN) from urea-amended the clay loam and sandy loam soil during the laboratory incubation period. The results show that the amount of organic-N mineralised in the clay loam soil was greater than sandy loam soil which may due to the clay loam soil had more organic matter.

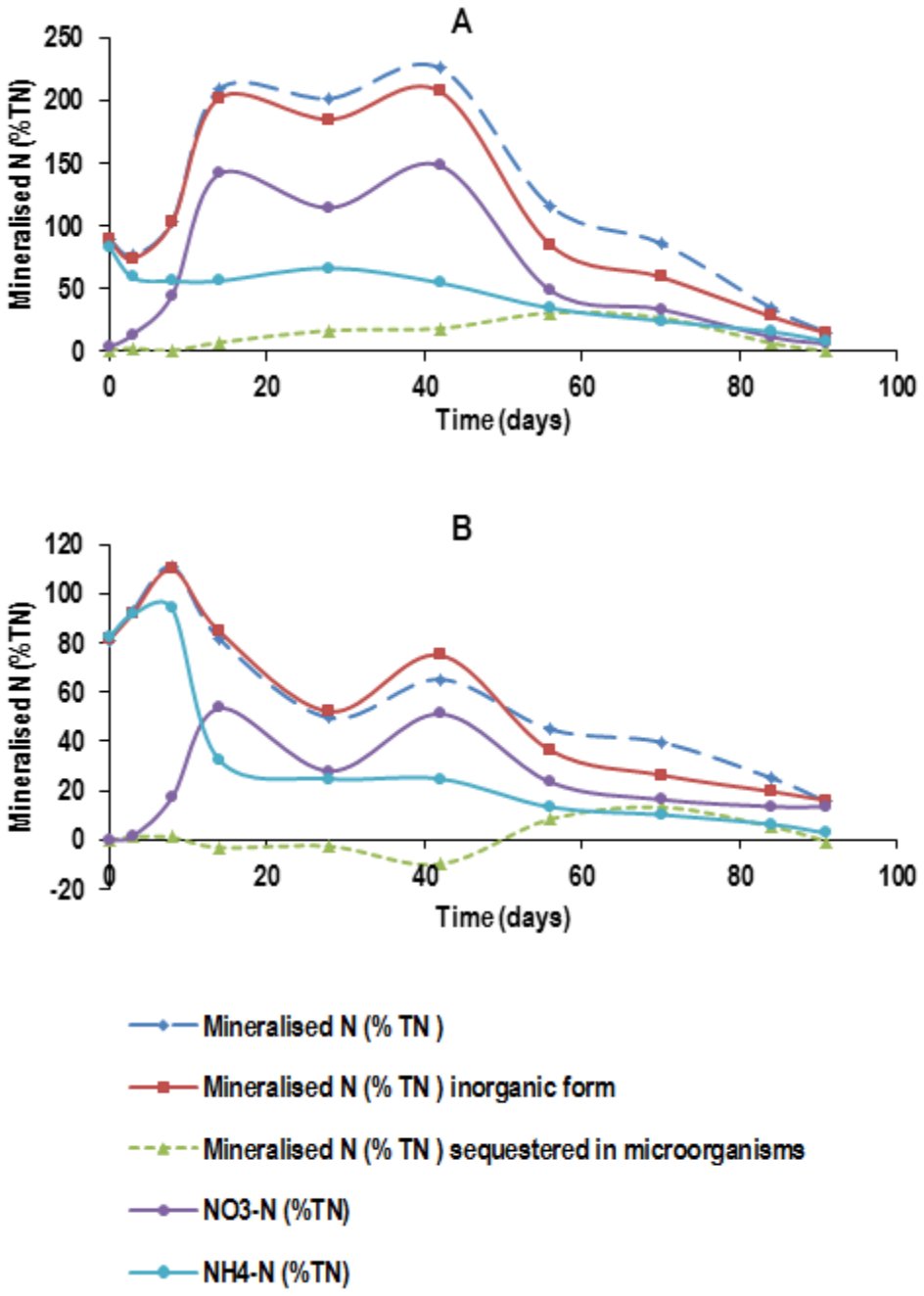

Figure 4.17. Mineral N (%TN added) from urea-applied to the clay loam (A) and sandy loam soil (B)

A similar amount of total N was added from urea as NH_4Cl (169 mg N kg^{-1}). The amount of ammonification released was double in sandy loam than clay loam soil, but it occurred on different. Therefore, the quantity of nitrification produced from urea-amended clay loam soil was higher which indicated that there was rapid nitrification on day 42 (Table 4.11).

In the sandy loam soil receiving urea, the total mineral N available was 111 % after 8 day following the application rate.

Table 4.11 Mineral N from urea-amended two soil types under laboratory incubation trail

Urea	Day of maximum mineralised N	% TNadded NH_4-N	% TNadded NO_3-N	% TNadded MBN	Mineralised N (% TN)
Clay loam soil	42	54 ± 2	148 ± 2	18 ± 1	225 ± 19
Sandy loam soil	8	94 ± 2	17 ± 1	1.1 ± 1	111 ± 14

4.3.2.9 Mineral N dynamics of ANDB in the clay loam soil

The rate of ammonification (NH_4-N) and nitrification (NO_3-N) processes of organic N in ANDB-applied to the clay loam soil soils is shown in Figure 4.18.

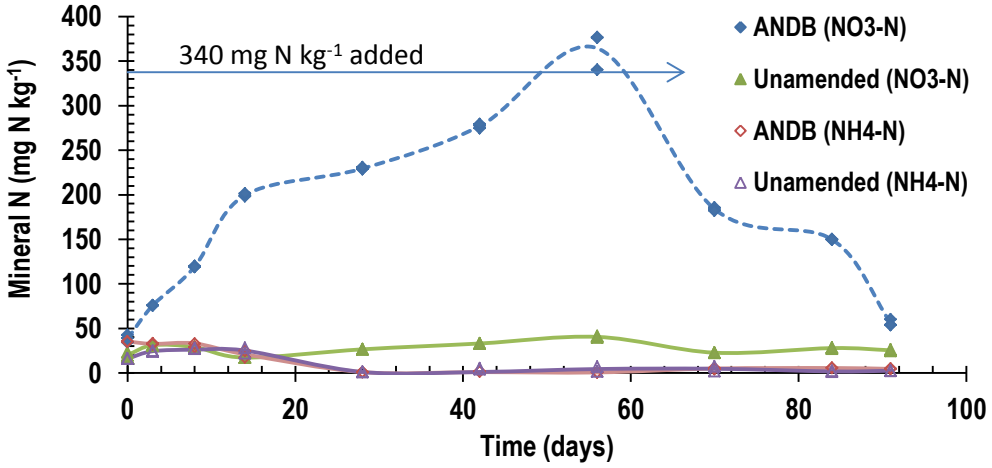

Figure 4.18. The effect of applying ANDB on NO_3-N and NH_4-N dynamics on the clay loam soil (n=3) (the connecting lines refers to the means of triplicate measurements plotted against times)

Production of mineral N (NH_4-N and NO_3-N) from ANDB

The ammonification process in ANDB and the unamended control clay loam soil initially decreased between days 1 – 28, and then remained relatively constant over 91 days with a corresponding increase in nitrification process.

In the clay loam soil treated with ANDB, the concentration of NO_3-N gradually increased to 199 mg N kg^{-1} between days 1 – 14. This represents the residual inorganic N and does not include the microbial biomass N. The quantity of NO_3-N determined was six times greater than the amount of NO_3-N produced in unamended control soil. However, NO_3-N concentration increased to 364 mg N kg^{-1} higher than the concentration of NO_3-N measured in the unamended control soil value on day 56. This is in agreement with other reported maximum mineralisation of organic-N in biosolids occurring within 56 days (Opperman et al., 1989, Powers, 1990, Smith et al., 1998b, Breedon et al., 2003, Pansu and Thuriès, 2003, Pu et al., 2012). The process of nitrification increased by 6.5 mg N kg^{-1} day^{-1} at 25 ˚C and soil moisture of 40 % WHC.

The maximum amount of NO_3-N determined in ANDB was greater than in the unamended control soil. The concentration of NO_3-N decreased between days 56 – 91. It has been shown that under anaerobic conditions when the pH is between 6 – 6.5 and the temperature is between 1.7 – 25, mineralised organic-N can be lost through denitrification (Smith et al., 1980, Fillery, 1983). The soils in the incubation experiment described in this chapter, fit within the range specified by Smith et al and Fillery et al and therefore a loss of mineralised organic-N can be expected.

4.3.2.10 Microbial biomass N and C

The concentration of MBN and MBC in ANDB-amended the clay loam soil over 91 days is presented in Figure 4.19.

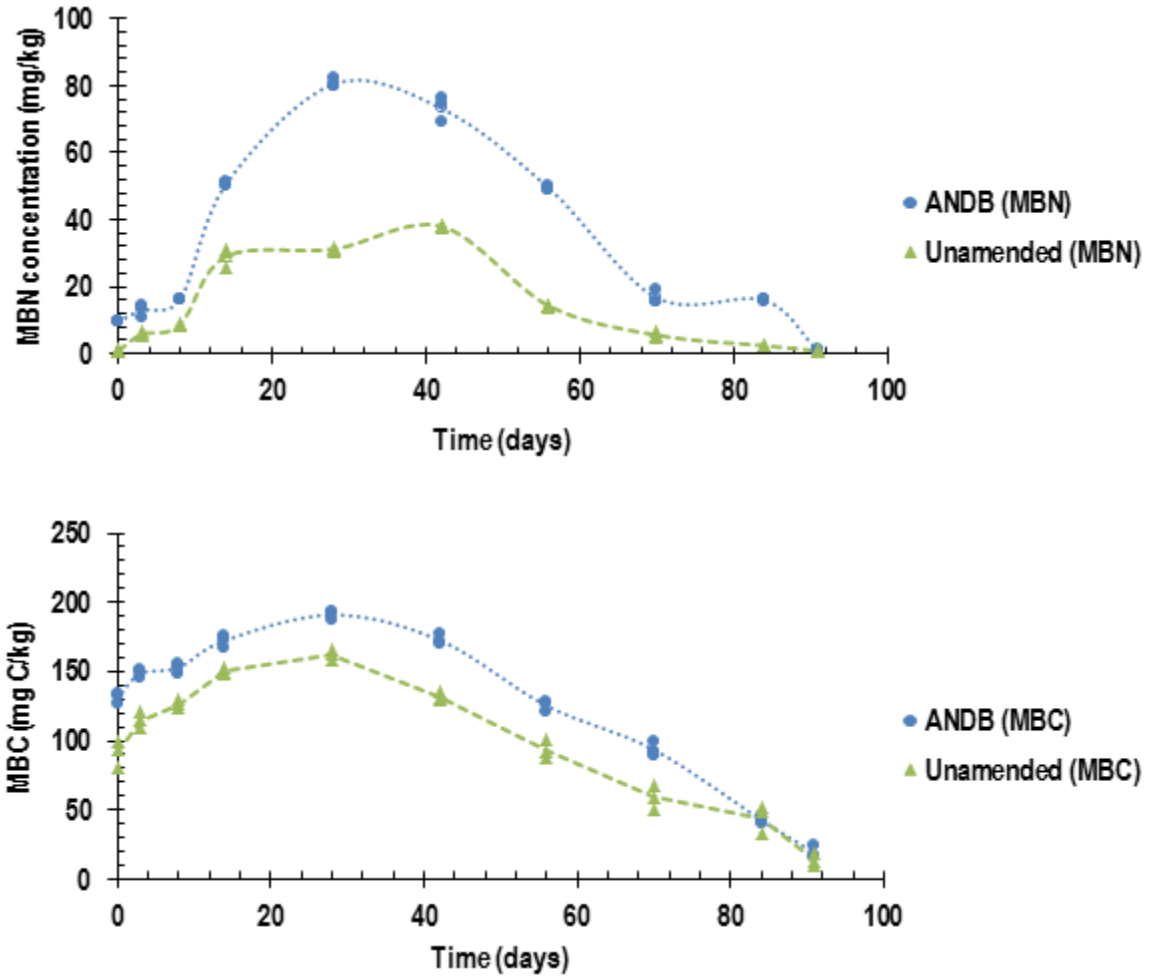

Figure 4.19. The influence of applying ANDB on MBN and MBC dynamics on clay loam soil (n=3) (the connecting lines refers to the means of triplicate measurements plotted against times)

The concentration of MBN in ANDB-amended clay loams soil increased between days 1 – 28, and then gradually decreased. The highest MBN concentration was 80 mg N kg^{-1} on day 28 whereas the levels of MBN in the unamended control soil increased to 31 mg N kg^{-1} at the same period. The quantity of MBN in ANDB was one and half times greater than the amount observed in the unamended control soil. As expected, the addition of organic matter stimulated soil microbial activities (Kaleem Abbasi et al., 2007). Furthermore, the MBN concentration was related to the organic C in the soil as reported in previous work (Thomsen et al., 2003).

In clay loam soil amended with ANDB, the highest concentration of MBC was 190 mg C kg^{-1} observed on day 28 compared to 162 mg C kg^{-1} found in the unamended control soil. Therefore, the level of MBC in ANDB was 17 % greater than the level

observed in the unamended control soil as expected from their organic matter content (Table 4.7). The greater microbial biomass values observed in this period can be attributed to an increase in the activity of microorganisms that are stimulated by high contents of nutrients and organic matter present in the biosolids (Jedidi et al., 2004, Rigby et al., 2009). MBN measured when MBC increased as a result of nitrifications.

Microbial biomass ratio

Microbial biomass C to N ratio in ANDB-amended the clay loam soil over 91 days is shown in Figure 4.20. The ratio of MBC: MBN was fluctuated during the incubation period and then increased by day 91.

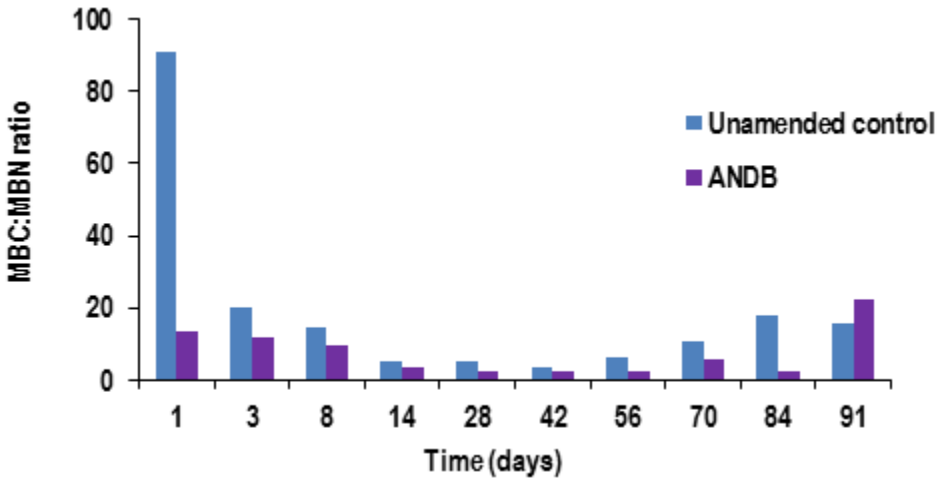

Figure 4.20. The ratio of microbial biomass C to N (mean, n = 3) in the ANDB-treated clay loam soil

4.3.2.11 Mineral N dynamics of ANDB in the sandy loam soil

The rate of ammonification (NH_4-N) and nitrification (NO_3-N) processes of organic-N in ANDB-applied to the sandy loam soil soils is shown in Figure 4.21.

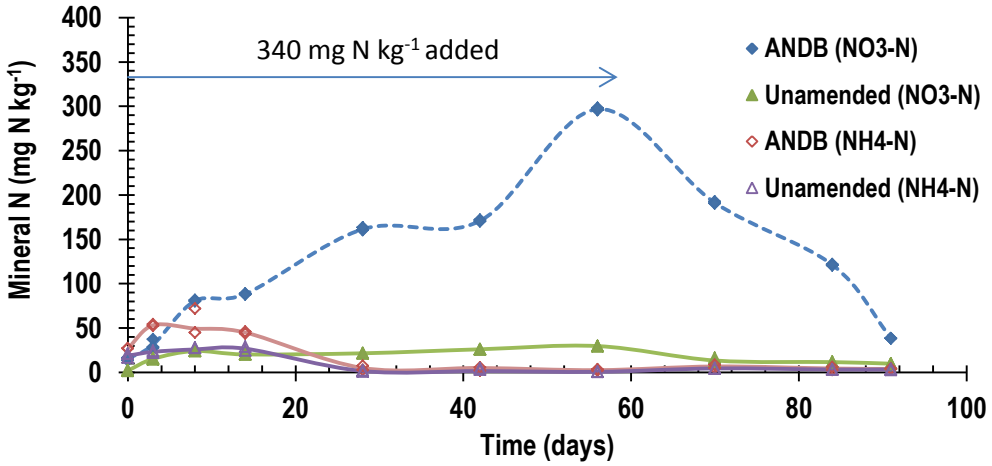

Figure 4.21. The effect of applying ANDB on NO_3-N and NH_4-N dynamics on the sandy loam soil (n=3) (the connecting lines refers to the means of triplicate measurements plotted against times)

Production of mineral N (NH_4-N and NO_3-N) from ANDB

The concentration of NH_4-N in ANDB-amended sandy loam soil increased by day 3 and then decreased over 91 days.

In the sandy loam soil receiving ANDB, NO_3-N concentrations doubled, increasing to 161 mg N kg^{-1} between days 1 – 28 compared to 8 mg N kg^{-1} observed in the unamended control soil. The NO_3-N level continued to increase reaching a maximum on day 56 (296 mg N kg^{-1}). This increase is consistent in the clay loam soil receiving ANDB and there was no effect of soil types receiving ANDB on the level of NO_3-N.

The greatest NO_3-N concentration in this material measured on day 56 was 296 mg N kg^{-1}. Thus, the nitrification process was increased by 5.28 mg N kg^{-1} day^{-1}. The determination of NO_3-N quantity in ANDB was eight times higher than the amount of NO_3-N detected in the unamended control soil. However, after day 56, the amount of NO_3-N decreased to lower concentration by day 91.

The statistical significance of the mineral N (NO_3-N and NH_4-N) concentrations was examined using ANOVA. There was a significant difference ($P < 0.001$) between the nitrification processes in ANDB-amended soils and the unamended control soils. However, there was no significant ($P > 0.05$) difference in the ammonification process between treatments.

4.3.2.12 Microbial biomass N and C

The concentration of MBN and MBC in ANDB-amended sandy loam soil over 91 days is presented in Figure 4.22.

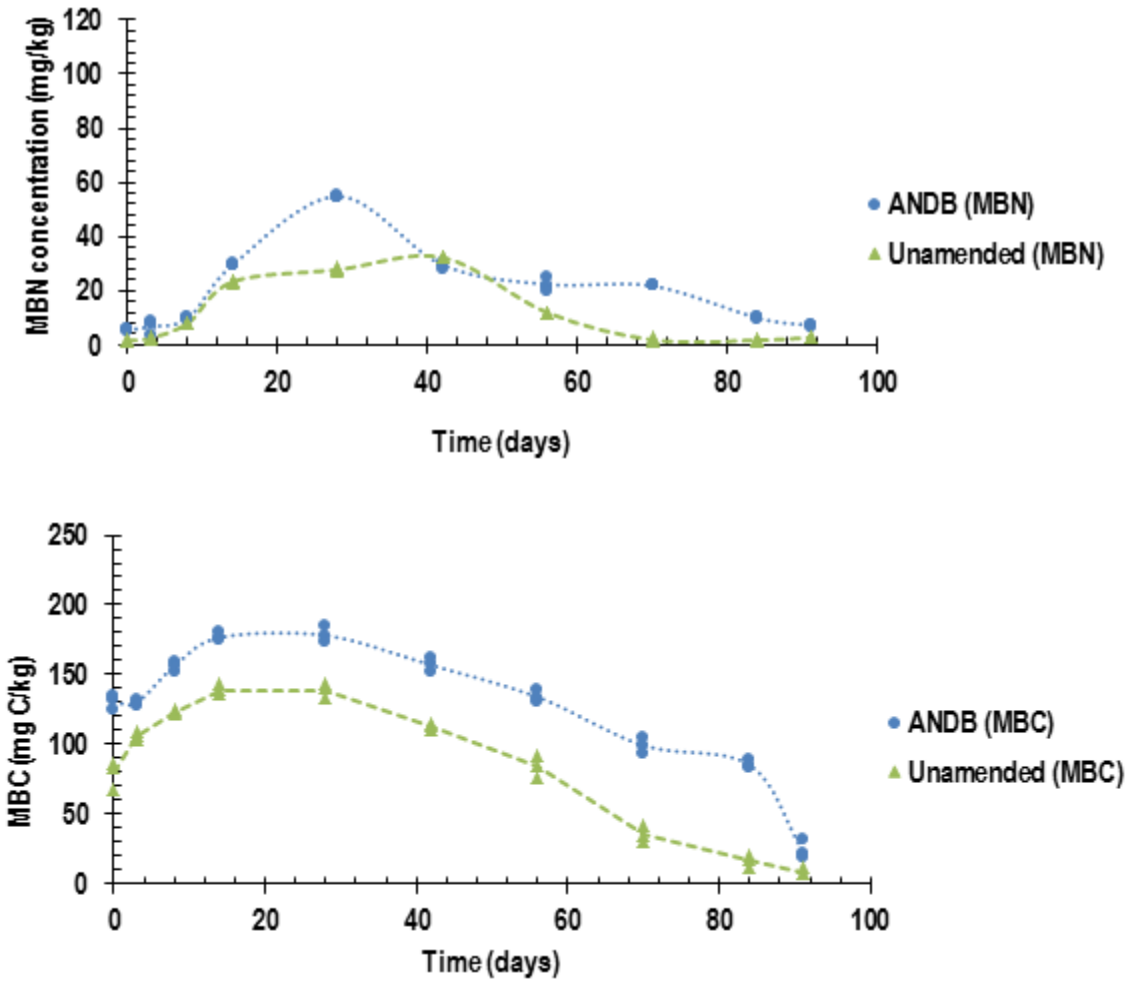

Figure 4.22. The influence of applying ANDB on MBN and MBC dynamics on sandy loam soil (n=3) (the connecting lines refers to the means of triplicate measurements plotted against times)

In sandy loam soil receiving ANDB, the level of MBN increased between days 1 – 28. The greatest concentration values were 55 mg N kg^{-1} in ANDB-amended soil compared to 28 mg N kg^{-1} observed in the unamended control soil. In this period, the mass of biosolids N immobilised was 96 % higher than the amount measured in the unamended control soil. However, the concentration of MBN decreased between days 28 – 91.

In the sandy loam soil receiving ANDB, the concentrations of MBC increased to 178 mg C kg^{-1} between days 1 – 28 compared to 139 mg C kg^{-1} measured in the unamended control soil. The quantity of MBC content in ANDB was 39 % higher than the amount obtained in the unamended control soil. The results obtained in the current study are similar to work done in the previous research (Chander and Brookes, 1991, Banerjee et al., 1997, Jahnel, 1997, Lopes, 2001) who confirmed that the application of sewage sludge favoured an increase in microbial biomass, as well as the activity of the soil microorganisms, probably because the activity of soil microorganisms was stimulated by the existence of organic matter from the sludge. However, the levels of MBC in ANDB, two fertiliser and unamended control decreased after day 28. A similar behaviour of MBN and MBC was observed in the clay loam soil but it was greater than sandy loam soil.

Microbial biomass ratio

Microbial biomass C to N ratio in ANDB-amended the sandy loam soil over 91 days is shown in Figure 4.23. The ratio of MBC: MBN was highest between days 1 – 3 and then decreased to lower values by day 28. However, the biomass ratio increased between days 42 – 91. The ratio of microbial biomass was greater in sandy loam than clay loam soil.

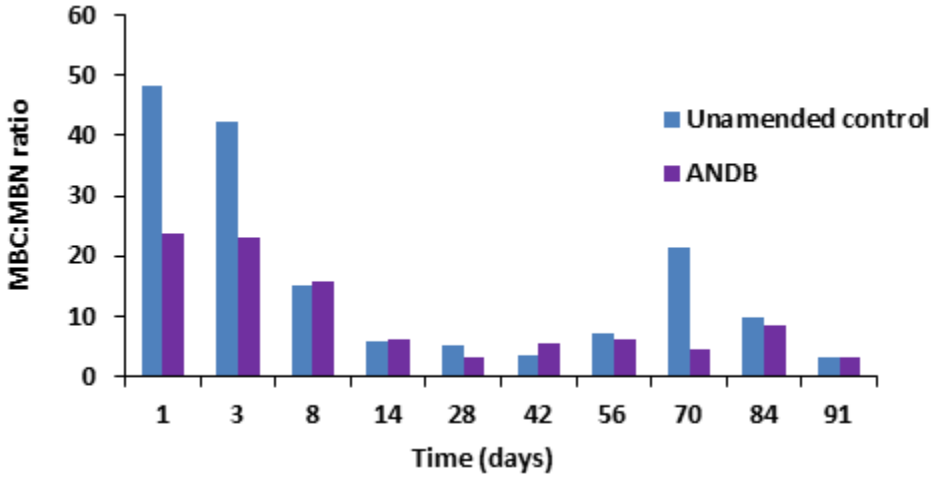

Figure 4.23. The ratio of microbial biomass C to N (mean, n = 3) in the ANDB-treated sandy loam soil

Comparison between two soil types amended with ANDB

The total mineral N observed from ANDB was about 100 % than total N added from ANDB in the clay loam soil and 84 % in the sandy loam soil on day 56 as presented in Figure 4.24.

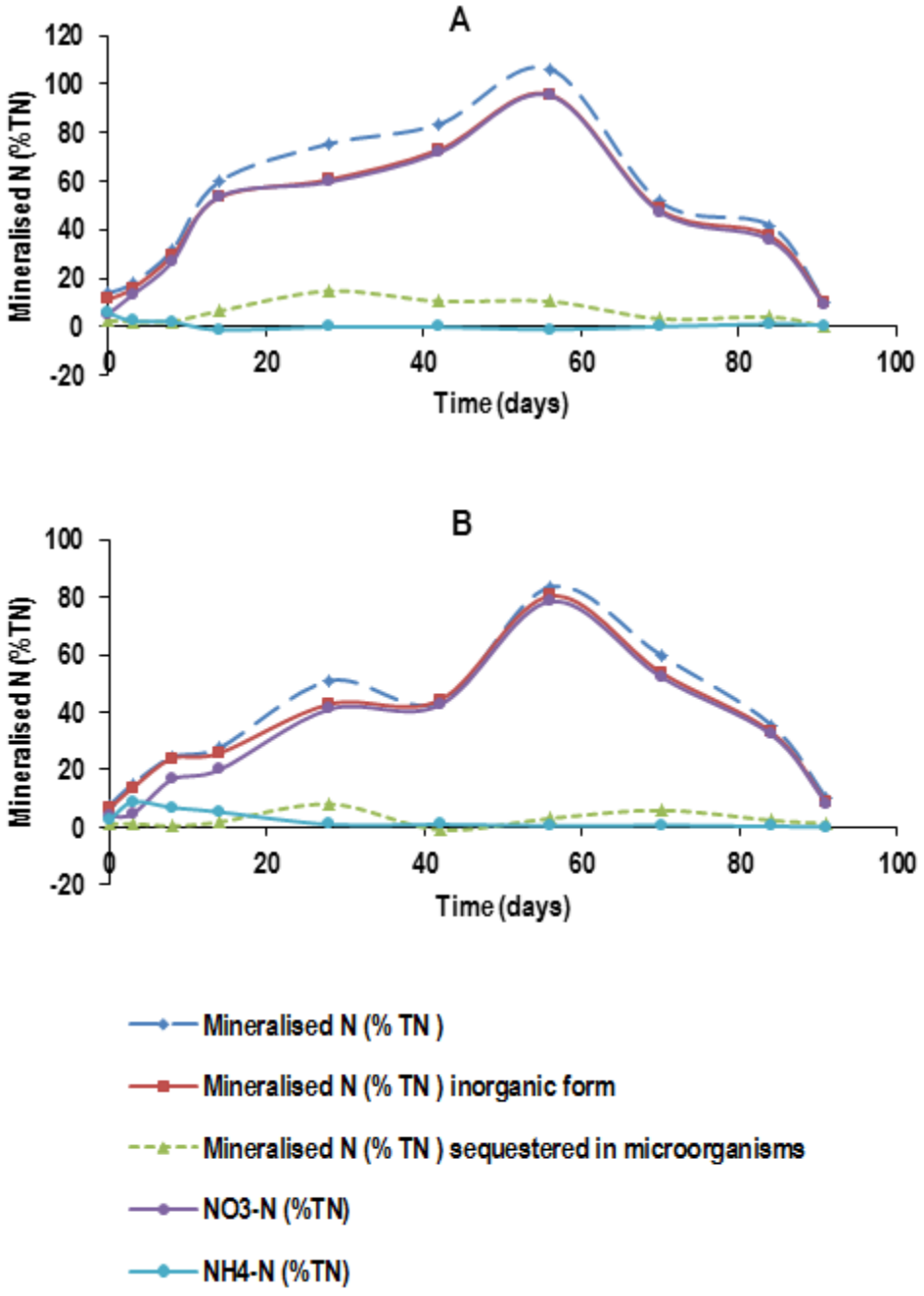

Figure 4.24. Mineral N (%TN added) from ANDB-applied to the clay loam (A) and sandy loam soil (B)

The amount of NH_4-N, NO_3-N and MBN as percentage of total N added from ANDB-amended two soil types shown in Table 4.12.

Table 4.12 Mineral N from ANDB-amended two soil types under laboratory incubation experiment

ANDB	Day of maximum mineralised N	% TNadded NH_4-N	% TNadded NO_3-N	% TNadded MBN	Mineralised N (% TN)	Organic-N mineralised (% org-N
Clay loam soil	56	1 ± 1	95 ± 3	10 ± 1	106 ± 19	105 ± 21
Sandy loam soil	56	1 ± 1	81 ± 3	3 ± 1	84 ± 9	78 ± 6

By day 56, the nitrification rate was greater in the clay loam soil than sandy loam soil; the microbial biomass N was greater in the clay loam soil. The amount of organic-N mineralised from ANDB-amended the clay loam soil was slightly greater than the N mineralisation observed in sandy loam soil receiving the same materials.

4.3.2.13 Mineral N dynamics of ADB in the clay loam soil

The change in the ammonification (NH_4-N) and nitrification (NO_3-N) processes of organic-N in ADB-applied to the clay loam soil soils is shown in Figure 4.25.

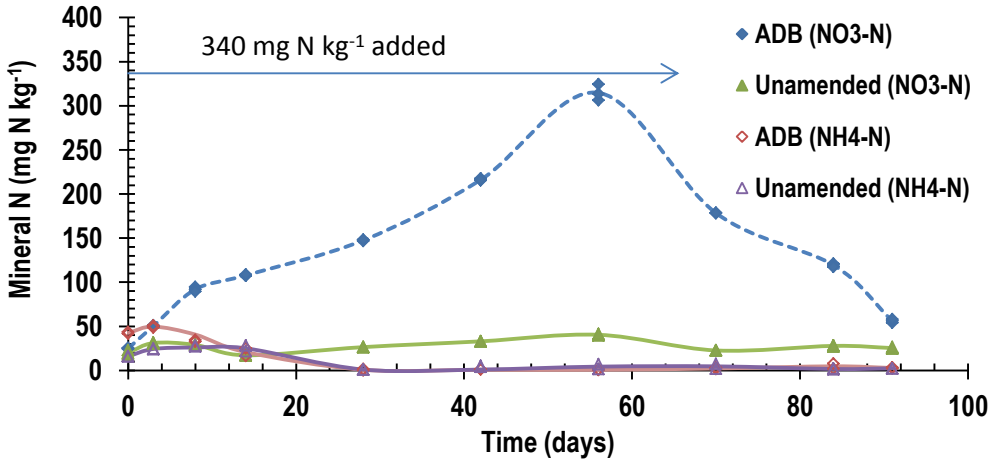

Figure 4.25. The effect of applying ADB on NO_3-N and NH_4-N dynamics on the clay loam soil (n=3) (the connecting lines refers to the means of triplicate measurements plotted against times)

Production of mineral N (NH_4-N and NO_3-N) from ADB

The concentration of NH_4-N in ADB and unamended control soil initially decreased between days 1 – 28, and then remained relatively constant over 91 days with a corresponding increase in NO_3-N concentration.

In the clay loam soil amended with ADB, the NO_3-N level increased to 91 mg N kg^{-1} and 26 mg N kg^{-1} in the unamended control soil between days 1 – 8. Moreover, a mount of NO_3-N produced in this material was three times greater than NO_3-N produced in the unamended control soil in this period. However, it appeared that the concentrations of NO_3-N in both treatments after day 8 onwards increased, reaching a maximum at day 56 of 324 mg N kg^{-1}, 39 mg N kg^{-1} for ADB and unamended control soil respectively. Furthermore, nitrification increased by 6 mg N kg^{-1} day^{-1}. Consequently, the quantity of NO_3-N produced from biosolids (ADB) was seven times higher than the mount of NO_3-N observed in the unamended control soil. During this period, the organic-N was transformed to inorganic-N forms with a corresponding decrease in NH_4-N. The levels of NO_3-N after day 56 decreased over 91 days.

The production of mineral N in the soils treated with biosolids was significantly higher ($P < 0.001$) than the in the control soils.

4.3.2.14 Microbial biomass N and C

The concentration of MBN and MBC in ADB-amended the clay loam soil over 91 days is presented in Figure 4.26.

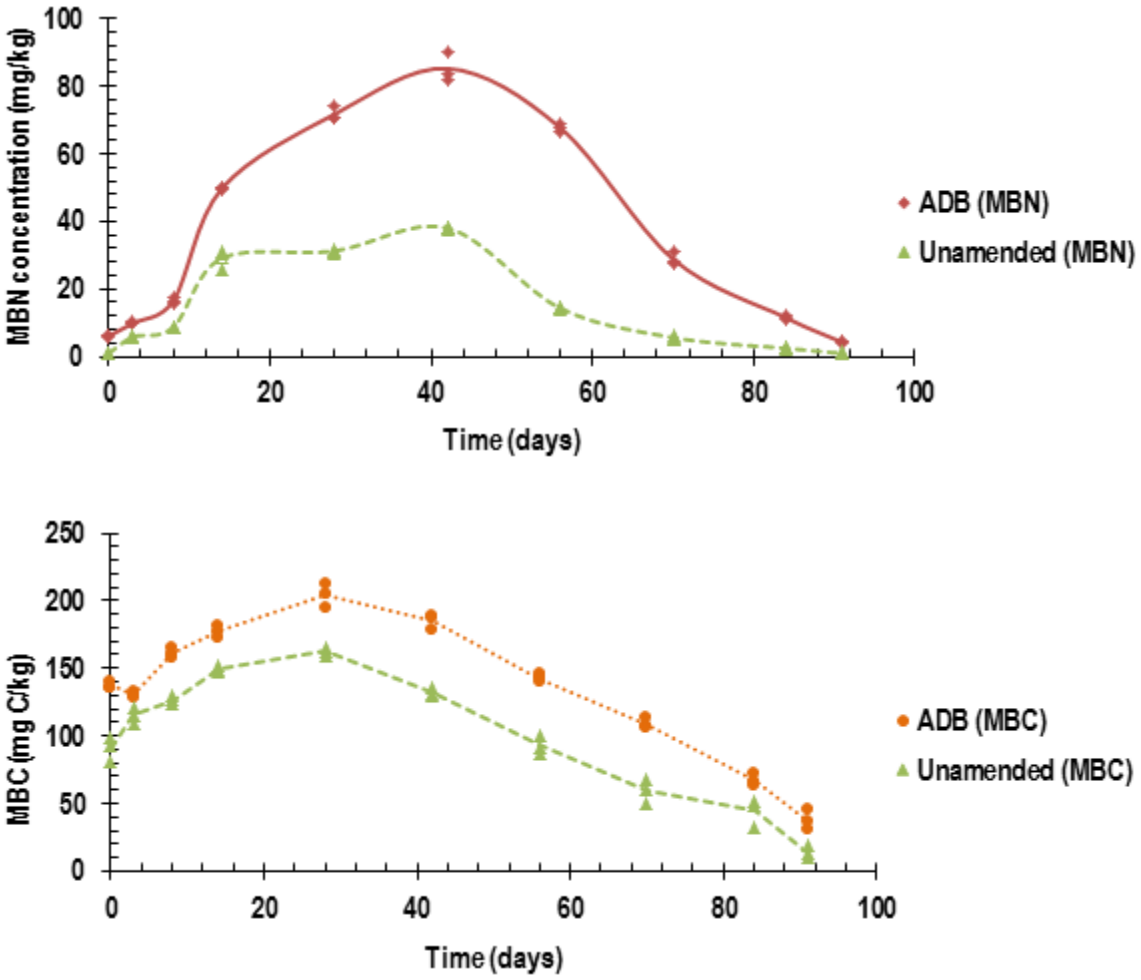

Figure 4.26. The influence of applying ADB on MBN and MBC dynamics on clay loam soil (the connecting lines refers to the means of triplicate measurements plotted against times)

The levels of MBN in ADB-amended a clay loam soil increased to 83 mg N kg^{-1} by day 42 which was double the corresponding amount observed in the unamended control soil.

The highest concentration of MBC in ADB was 195 mg C kg^{-1} observed on day 28 compared to 162 mg C kg^{-1} found in the unamended control soil. Therefore, the level of MBC in ADB was 18 % greater than the level observed in the unamended control soil as expected from their organic matter content. The increase the amount of MBN released as a result of an increasing nitrification process. This is consistent with urea and ANDB-amended the clay loam soil as described above.

Microbial biomass ratio

Microbial biomass C to N ratio in ADB-amended the clay loam soil over 91 days is shown in Figure 4.27. The ratio of MBC: MBN was highest on day 1 and then decreased to a minimum by day 56. However, the MBC: MBN ratio slightly increased again after day 70.

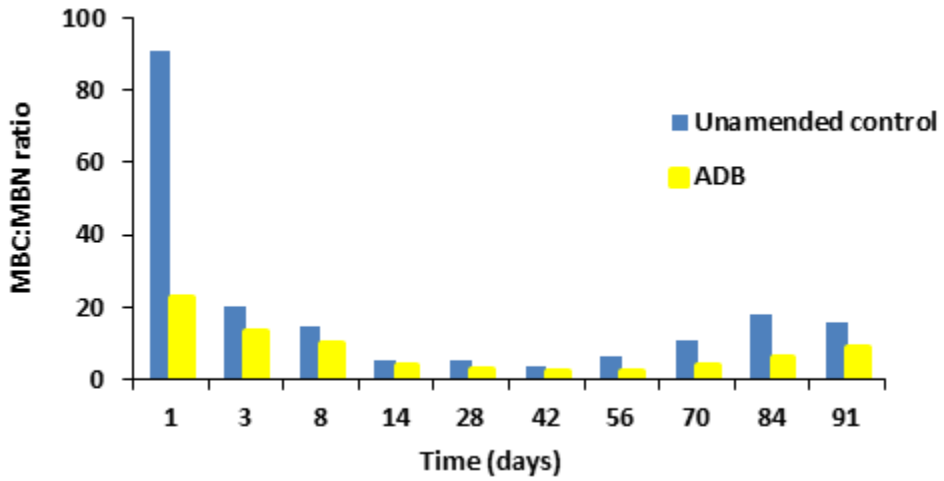

Figure 4.27. The ratio of microbial biomass C to N (mean, n = 3) in the ADB-treated clay loam soil

4.3.2.15 Mineral N dynamics of ADB in the sandy loam soil

The rate of ammonification (NH_4-N) and nitrification (NO_3-N) processes of organic-N in ADB applied to the sandy loam soil soils is shown in Figure 4.28.

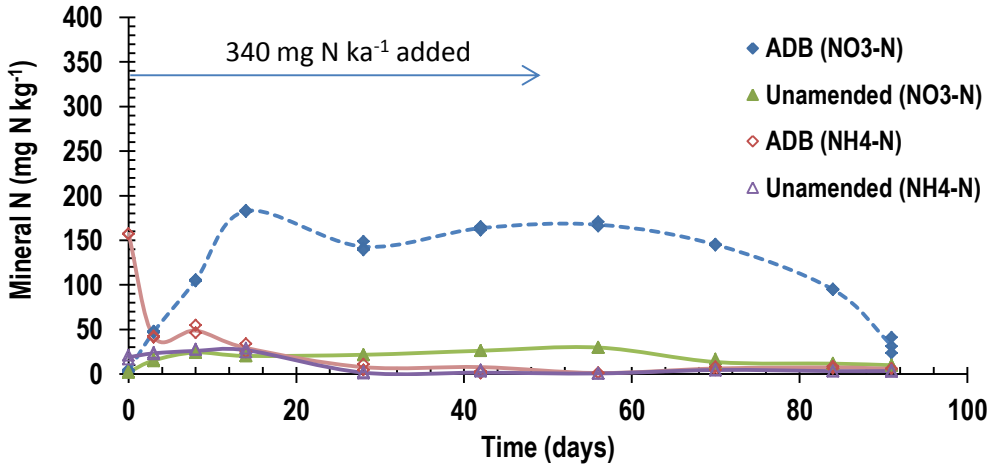

Figure 4.28. The effect of applying ADB on NO_3-N and NH_4-N dynamics on the sandy loam soil (n=3) (the connecting lines refers to the means of triplicate measurements plotted against times)

Production of mineral N (NH_4-N and NO_3-N) from ADB

The level of NH_4-N in ADB drops down between days 1 – 3 and then decreased. There was no significant difference ($P > 0.05$) in the NH_4-N concentrations between the ADB and unamended control soil.

The concentration of NO_3-N measured in ADB-amended sandy loam soil, increased from the initial concentration of 4 mg N kg^{-1} to 182 mg N kg^{-1} on day 14. it is equivalent of a nitrification rate of 13 mg N kg^{-1} day^{-1}, which is consistent with the rapid nitrification observed within 20 days in an incubation experiment conducted by Smith et al. (1998b). Hence, the mass of NO_3-N produced by applying ADB to the clay loam soil was greater than the amount of NO_3-N found in the unamended control soil. However, the level of NO_3-N in ADB decreased between days 14 – 28 by 142 mg N kg^{-1}. The levels of NO_3-N slightly increased from day 28 to day 56 and then gradually decreased to the end of the experimental period. The NO_3-N concentrations in the unamended control soil remained steady during the incubation period.

The mineral N concentration in ADB and unamended control soil were tested by ANOVA. There was a significantly higher ($P < 0.001$) release of NO_3-N from the ADB-amended soil types than the unamended controls.

4.3.2.16 Microbial biomass N and C

The concentration of MBN and MBC in ADB-amended the sandy loam soil over 91 days is presented in Figure 4.29.

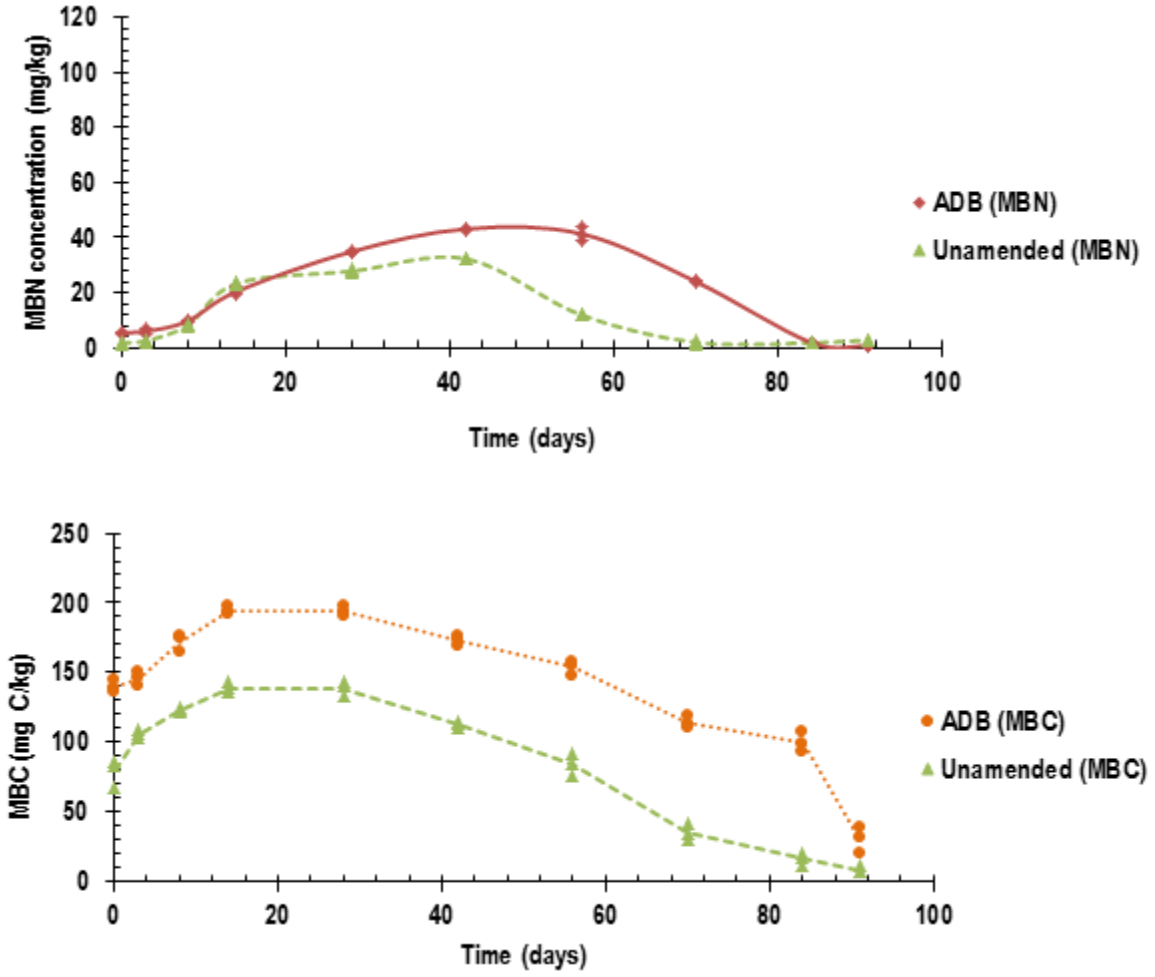

Figure 4.29. The influence of applying ADB on MBN and MBC dynamics on sandy loam soil (n=3) (the connecting lines refers to the means of triplicate measurements plotted against times)

The concentration of MBN in ADB was between 5 – 42 mg N kg^{-1} from days 1 – 42. The levels of MBN then increased to 44 mg N kg^{-1} by day 56. However, the concentration of MBN in ADB-treated the sandy loam was lower than in the clay loam soil receiving the sane biosolids. The quantity of MBN was two times higher than the amount observed in the unamended control soil. After day 56 onwards the MBN dynamics decreased.

The levels of MBC reached a maximum value of 191 mg C kg^{-1} in ADB-amended sandy loam soil on day 14. At this point, the amount of MBC content in ADB was 39 % higher than the mass of MBC content observed in the unamended control soil. The levels of MBC in ADB decreased after day 14 until the end of incubation period.

Analysis of variance (ANOVA) indicated that there were significantly ($P < 0.05$) higher concentrations of MBC observed in ADB-amended clay loam and sandy loam soil than in the unamended control soils.

Microbial biomass ratio

Microbial biomass C to N ratio in ADB-amended the sandy loam soil over 91 days is shown in Figure 4.30. The ratio of MBC: MBN decreased gradually from days 1 – 70 and then increase to the maximum between days 82 – 91. It might be due to the growth of fungi.

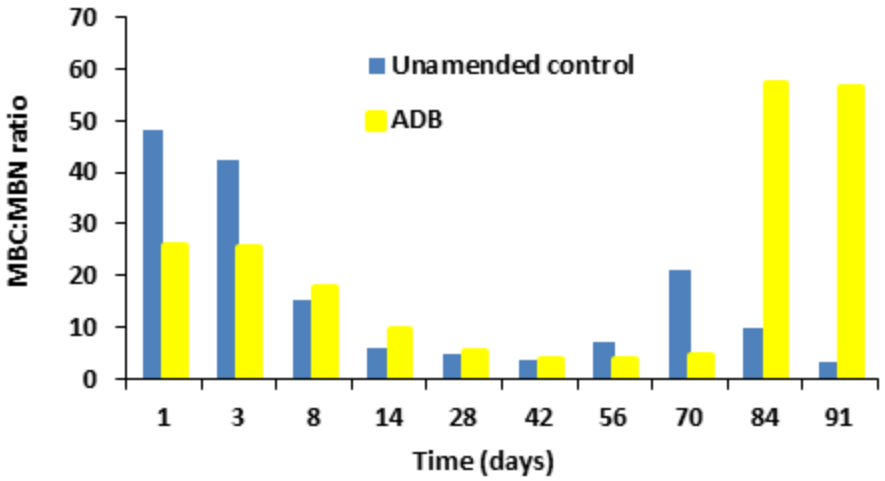

Figure 4.30. The ratio of microbial biomass C to N (mean, n = 3) in the ADB-treated sandy loam soil

Comparison between two soil types amended with ADB

The amount of total N mineralised from ADB-treated the clay loam soil was approximately 93 % than the amount of total N added by day 56 (Figure 4.31). However, the amount of total mineral N added from ADB-amended sandy loam soil was approximately 49 % on day 8.

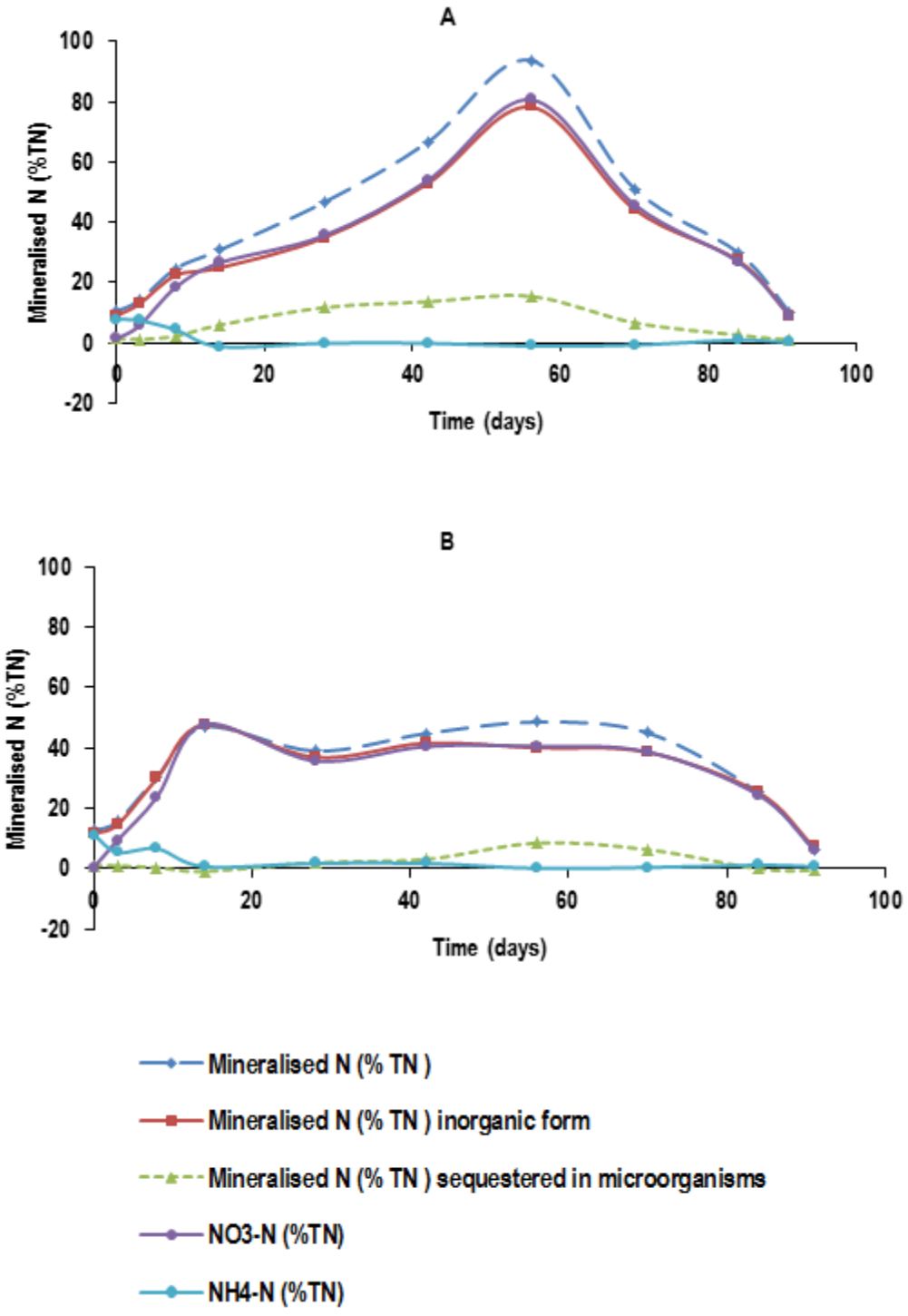

Figure 4.31. Mineral N (%TN added) from ADB-applied to the clay loam (A) and sandy loam soil (B)

The quantities of NH_4-N, NO_3-N and MBN as a percentage of total N added from ADB-amended two soil types are presented in Table 4.13.

Table 4.13 Mineral N from ADB-amended two soil types under laboratory incubation trail

ADB	Day of maximum mineralised N	% TNadded NH_4-N	% TNadded NO_3-N	% TNadded MBN	Mineralised N (% TN)	Organic-N mineralised (% org-N
Clay loam soil	56	-2 ± 1	80 ± 3	16 ± 3	94 ± 4	102 ± 3
Sandy loam soil	56	0.1 ± 1	41 ± 2	9 ± 2	49 ± 3	41 ± 3

The nitrification rate in the clay loam soil was double than the amount of NO_3-N observed in the sandy loam soil which indicated to rapid mineralisation and nitrification process on day 56. Also, the amount of organic-N mineralised observed in the clay loam soil was double than sandy loam soil after 56 days following the application rate. The proportion of organic-N mineralised in ADB-amended the clay loam soil was around to 102 % and 41 % in the sandy loam soil.

Comparing between ANDB and ADB, the fraction of organic-N mineralised was greater in ANDB than ADB which indicated that the ANDB had more organic-N.

4.3.3 Effect of amendments on mineral N

The nitrification process in the urea-amended soils was double that of the NH_4Cl amendment on the same soils. This might be because the amount of NH_4-N in NH_4Cl treatment converted to NH_3-N during the experiment established which indicated that there was loses of N through volatilisation process.

The amount of organic-N in ANDB was greater than ADB which indicated that there is likely to be more N available in ANDB (Table 4.7) than in ADB. The effect of biosolids type on the availability of mineral N on the clay loam and sandy loam soils are shown in Figure 4.18, Figure 4.21, Figure 4.25 and Figure 4.28. On both soils, the maximum amount of NO_3-N mineralised from ANDB was greater than from ADB, but the difference was much more marked in the sandy soil (16% versus 77% of TN).

4.3.4 Effect of soil type on mineral N

With the application of NH_4Cl and urea, the amount of NO_3-N produced in the fertile clay loam soil was one and half times greater than the less fertile sandy loam soil (Figure 4.11 and Figure 4.14). This is an expected result since the nitrification

process is enhanced by high organic matter since it stimulates soil microbes to breakdown the organic-N forms in soil to inorganic-N forms (Keeney and Nelson, 1982, Ajwa and Tabatabai, 1994, Eriksen et al., 1999, Smith and Durham, 2002, Morris et al., 2003, Huang and Chen, 2009).

By day 56, NO_3-N produced from ANDB in the amended clay loam soil was 23 % greater than the corresponding of NO_3-N nitrified on sandy loam soil. The observed NO_3-N mineralised from ADB amended clay loam soil was 88 % higher than NO_3-N produced in ADB amended sandy loam soil. This indicates that the organic-N was more rapidly mineralised in more fertile clay loam soil than in the less fertile sandy loam soil (Table 4.7). Highly fertile soils encourage microbial activity (Silva et al., 2005a, Abbasi and Khizar, 2012, Ryals et al., 2013) which would explain the difference in response to the biosolids on the two soils.

The differences observed in NO_3-N accumulation between the two soils may be attributed to soil heterogeneity (Davidson and Hackler, 1994). For instance, the initial delay in the production of NO_3-N in the clay loam soil could be due to the temporary fixation of $NH4^+$ ions by clay minerals (Tester et al., 1977, Feigenbaum et al., 1994). The accumulation behaviour of mineralised N from organic wastes observed in this study were similar to those reported by Chae and Tabatabai (1986b) in soils with properties comparable to those studied herein.

There are various losses of gaseous N through denitrification or ammonia (NH_3-N) volatilisation which can potentially cause significant errors in evaluations of N availability of organic-N biosolids determined in laboratory incubation experiments, depending upon the experimental conditions. Major losses of N through denitrification process from incubated soils have been reported in previous research (King, 1973, Hsieh et al., 1981), particularly for undigested biosolids rich in available substrate C (Epstein et al., 1978, Parker and Sommers, 1983, Garau et al., 1986, Serna and Pomares, 1992), and the extent of N loss by this cause can increase with the application rate of biosolids (Lindemann and Cardenas, 1984, Adegbidi and Briggs, 2003a, Hseu and Huang, 2005).

4.3.5 Effect of biosolids and soil types on MBN

The concentration of MBN in both fertiliser (NH_4Cl and urea) amended two soil types was greater in the clay loam than sandy loam soil.

On the clay loam soil, the quantity of N immobilised from ANDB was 13 % greater compared with the mass of MBN in ADB on day 28. But then, the amount of MBN observed in ADB was 16 % higher than the amount of MBN content obtained in ANDB on day 56. This may be due to the stability of organic matter on aerobically digested biosolids having an impact on MBN dynamics. The increase in microbial population as a function of biosolids application may be due to the presence of easily degradable organic components detected in organic residues which are necessary for microbial growth (Sakamoto and Oba, 1991, Lima et al., 1996).

In comparison between ANDB and ADB amended sandy loam soil, the amount of biosolids N in ANDB was 57 % higher than the corresponding to content of MBN in ADB on day 28 (Figure 4.22 and Figure 4.29). This indicated that the ANDB had higher organic-N than ADB (Table 4.7). On the other hand, the extent of MBN in ADB was 48 % greater compared with the amount of MBN in ANDB on day 56. This is consistent with an increasing of maximum values of the NO_3-N concentration observed on the same day (56).

Two soil types amended with ANDB as shown in Figure 4.19 and Figure 4.22. The amount of N immobilised from the biosolids was 45 % greater in clay loam than sandy loam soil on day 28. Similar MBN pattern observed when ADB amended clay loam and sandy loam soil. It was approximately two times higher in the clay loam than sandy loam soil.

4.3.6 Effect of biosolids and soil types on microbial biomass C

As expected, the addition of organic matter to the soil had a positive influence on the soil microbial population (Jedidi et al., 2004, Fernández et al., 2007, Plaza et al., 2007, Cayuela et al., 2008, Rigby et al., 2009).

The concentrations of MBC in both fertilisers were greater in the clay loam soil than sandy loam soil.

In both biosolids-amended soils, the concentrations of MBC in were greater than in either unamended control soil types. This might be due to the high amount of readily mineralisable organic matter which may be expected from both biosolids that encourage microbial biomass stimulation (Jedidi et al., 2004).

The MBC concentrations in the clay loam soil amended with ANDB and ADB were greater than the concentration of MBC in sandy loam soil amended with similar materials, indicative of the greater soil organic matter in the clay loam. The variability in microbial biomass might also be related to the soil textures used in the studies (Brookes and McGrath, 1984, Dar, 1996). It has often been observed that there is a negative relationship between clay content and rate of breakdown and the soil various texture (Day et al., 1978, Recous et al., 1990). Organic amendments tend to decompose more slowly in clay soils than sandy soils which has been attributed to greater immobilisation or occlusion of C and lower aeration in the soils (Goyal et al., 1992). The difference in biological activity between soils seemed to be associated to the difference in their initial readily-available C and it did not significantly affect the rate of biosolids decomposition because N concentration was not the limiting aspect (Hadas et al., 1996). This is observed in this study in the clay loam soil.

Comparing between two soil types receiving two different biosolids types indicated that the concentration of MBC in the clay loam soil was greater than the corresponding MBC concentration found in the sandy loam soil. These results demonstrated that the clay loam soil is in a better condition for microbial activity compared with the less fertile sandy loam. Soil microbes are the existing part of soil organic matter and show critical roles in soil C and N cycling and ecosystem functioning (Doran, 1987). They assist as both source and sink of plant nutrients (Dalal, 1998). The activity of soil microbes greatly impacts short term dynamics and long-term stability of organic matter in soil. Bastida et at., (2008) concluded that microbial biomass and activity of degraded semiarid soils can be enhanced by the addition of organic materials of differing degrees of stabilization (compost and sewage sludge).

Microbes are typically C-limited in agricultural land soils (Smith and Paul, 1990), and microbial biomass and activities are thus closely associated to labile organic C in soil. Soil microbial biomass and activity react sensitively to changes in organic C levels or quality caused by agronomic practices and other disturbances (Powlson et al., 1987, Lundquist et al., 1999, Tu et al., 2006). High microbial activities are characteristically combined to high C turnover and CO_2 release; thus management practices that reduce microbial access to organic matter should promote soil C accumulation.

4.4 Conclusion

A laboratory incubation experiment was established with a clay loam and a sandy loam soil amended with anaerobically digested biosolids (ANDB), aerobically digested biosolids (ADB) and two fertilisers (urea and NH4Cl) as references. The main purpose of this experiment was to investigate the change of mineral N movement throughout the incubation period in two biosolids-amended soils using two types of soils under controlled temperature (25 °C) and moisture content (40 %). More specifically, it was to calculate the rate of mineralisation N of the two fertilisers and two biosolids during a three month incubation period (91 days). In addition, microbial biomass C and N were measured to determine the amount of N immobilised in the biomass.

N mineralisation of the two biosolids reached its peak around day 56. The maximum values for the two fertilisers occurred earlier. After the maximum was reached there was a reduction NO_3-N assumed to be a result of the denitrification process.

The findings of the laboratory incubation experiment indicate that the greatest value of net NO_3-N mineralised in ANDB and ADB-amended a clay loam soil were 364 and 324 mg N kg^{-1} respectively on day 56. In the sandy loam soil amended with ANDB, the highest net NO_3-N released was 296 mg N kg^{-1} on day 56, while 182 mg N kg^{-1} net NO_3-N produced from ADB-applied in a sandy loam soil on day 14.

The concentrations of MBN and MBC in ANDB were greater than the amount of MBN in ANDB-amended two soil types. Also, there was a great microbial biomass N and C in the clay loam soil than sandy loam soil.

For the ratio of microbial biomass C to N, there was a high value observed on all treatment in both soil at the beginning and then C/N decreased. Among all these treatment, there was an increase in the MBC: MBN which may be due to the growth of fungi during this period.

The greatest proportion of organic-N mineralised from the organic-N contained in ANDB was 105 and 78 % in the clay loam and sandy loam soil respectively which was greater than the amount of organic-N contained in ADB-amended the clay loam (102 %) and sandy loam soil (40 %).

The in-situ incubation approach may help provide site-specific estimates of N mineralisation from land-applied biosolids. However, it may only be an approximation to what is likely to happen under field conditions. In the next chapter, the results from a field trial in which the experimental design in this incubation experiment, was used.

5

5 An Investigation into the Mineralisation Rates of Nitrogen and Immobilisation of Carbon and Nitrogen in Soil Amended with Biosolids under Field Conditions: no vegetation

5.1 Introduction

The results from laboratory incubation experiments described in Chapter 4 gave an estimation of how long it takes for the organic N to be mineralised and the percentage of N mineralisation from these materials. However, in the field, temperatures are variable as are moisture content and sunlight, so a field experiment was conducted to check the applicability of the results from the laboratory experiment to the field (Gilmour et al., 2003, Bowden et al., 2007, Pu et al., 2012). In this Chapter, the same experimental design was used on two field sites as described in Chapter 4, with the same soils, biosolids and fertilisers. The experiment was repeated with and without vegetation growing on the experimental plots. This chapter reports on the experiment without vegetation. The sites were kept free of plants by hand weeding every few days.

As in the incubation experiments, the biosolids-amended soil samples were removed at interval times of 1, 8, 15, 22, 31, 46, 61, 77 and 107 days to determine NO_3-N, NH_4-N, MBC and MBN as described in chapter 4.

The specific aims of the work reported in this chapter were to:

- investigate the influence of soil type and biosolids type on N mineralisation and immobilisation of C and N under field conditions
- evaluate the effect of environmental factors such soil temperature and soil moisture on N mineralisation and immobilisation of C and N

- calculate the mineralisable portion of the organic N in the two biosolids types applied on to two soil types in the field.

5.2 Experimental design and treatments

A field incubation experiment was established on 05th May 2011 at LA and 7th May at MRWP. Biosolids and fertilisers were applied at the same application rates as used in the incubation experiment in Chapter 4. A randomized blocked design was used with eight treatments in triplicate as shown in in Figure 5.1.

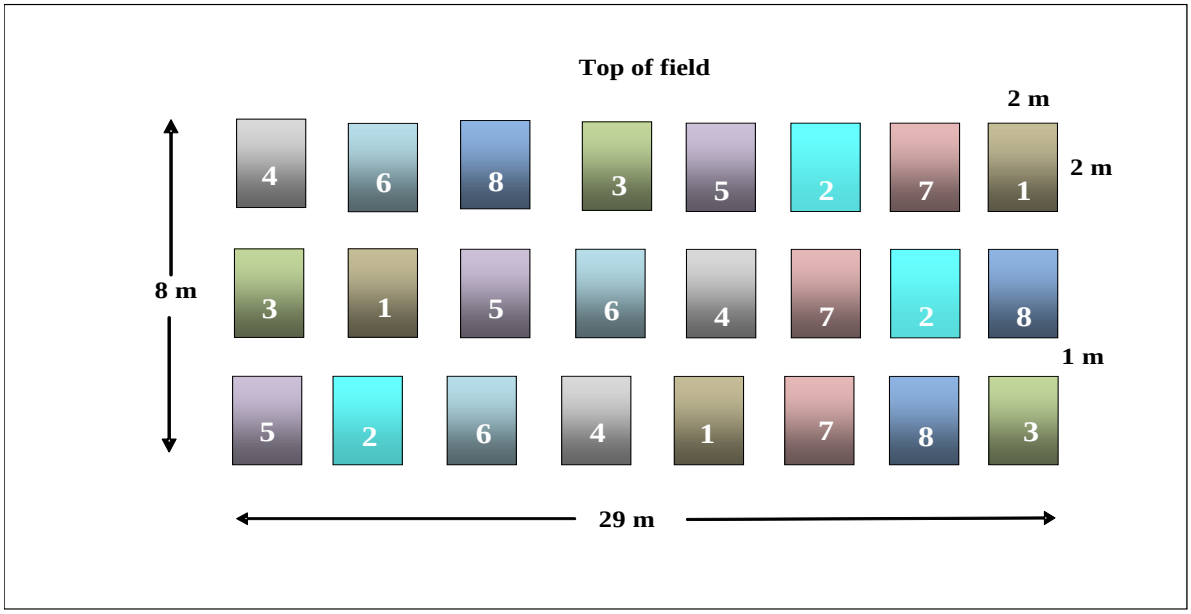

Figure 5.1. Design of mineralisation experiment in field site

Description of the numbers assigned for each treatments:

1. Unamended control plots with no vegetation,
2. Unamended control with planted ryegrass
3. Melton Recycled Water Plant biosolids applied at a rate of 15 t ds ha^{-1} with no vegetation
4. Melton Recycled Water Plant biosolids applied at a rate of 15 t ds ha^{-1} with planted ryegrass
5. Barwon Water biosolids applied at a rate of 15 t ds ha^{-1} with no vegetation

6. Barwon Water biosolids applied at a rate of 15 t ds ha^{-1} with planted ryegrass
7. Urea fertilizer supplied at a rate of 250 kg ha^{-1} with no vegetation
8. NH_4Cl fertilizer supplied at a rate of 250 kg ha^{-1} with no vegetation

The application rates of biosolids and fertiliser were calculated based on N concentration as presented in Table 5.1.

Table 5.1 The application rates calculated based on N concentration

Treatments	t ds ha^{-1}	kg N ha^{-1}	biosolids or fertiliser 4 m^{-2}	Total N added mg kg^{-1}
MRWPs with ANDB	15	510	9.1 kg	342
MRWPs with ADB	15	510	20.0 kg	342
LAs with ANDB	15	510	9.1 kg	342
LAs with ADB	15	510	20.0 kg	342
MRWPs with Urea	-	250	217 g	110
MRWPs with NH_4Cl	-	250	385 g	110
LAs with Urea	-	250	217 g	110
LAs with NH_4Cl	-	250	385 g	110

MRWPs = Melton Recycled Water Plant site, ANDB = Anaerobically digested biosolids, LAs = Lara site, ADB = Aerobically digested biosolids

The experiment was partitioned as per the design described in Figure 5.1. The size for each plot was 2 m × 2 m (Figure 5.2 and Figure 5.3). Biosolids and fertilizers were weighed into plastic containers using a top loading balance, and incorporated to a soil depth of 15 cm.

Figure 5.2. Experimental plots at the Melton Recycled Water Plant site

Figure 5.3. Experimental plots at the Lara site

To examine the influence of crop cover on N mineralisation, perennial ryegrass (*Lolium perenne*) was used as an indicator crop and sown at a seeding rate of 200 kg ha^{-1} leaving 20 cm space between each row for each plot. The results from this section of the study are shown in Chapter 6.

Temperature data loggers (Tiny Tag Transit 2 Temperature Logger - 40ºC to +70 C) were inserted to a soil depth 5 cm in randomly selected plots (Figure 5.4).

Figure 5.4. Temperature data loggers used in the field, the arrow indicates the location where the data loggers were inserted in the plots at both sites

The loggers were allowed to stay in the soil for 4 months and after 120 days were removed from each plot. The recorded hourly temperatures were imported into an Excel spread sheet. All data were averaged, to provide average weekly temperatures for the sites. Soils were sampled on a weekly basis for moisture content.

Three replicate samples from each of the treatments were taken throughout field experimental period at days 1, 8, 15, 22, 31, 46, 61, 77, and 107. Five sample plugs (15 cm depth) from each of the experimental plots were combined in one plastic bag, homogenised and transported to the laboratory (Figure 5.5 and Figure 5.6). Biosolids-amended soil samples were analysed for NO_3-N and NH_4-N (Chapter 3, Section 3.5). The standard calibration curves constructed for the calculation are shown in Table 5.2. The samples analysed for MBC and MBN (Chapter 3, Section 3.6) and equations constructed to determine the quantities of microbial biomass are shown in Table 5.3.

Table 5.2 Analysis data for NO_3-N in all treatment in each day (field incubation experiment)

Time (days)	Date of analysis	slope	intercept	R^2
1	15 March 2011	365	35	0.998
8	16 March 2011	373	11	0.998
15 22	22 March 2013	318	12	0.999
31 46	24 March 2012	315	18	0.999
61 77 107	28 March 2012	318	15	0.999

(A fresh setting calibration was made for each day)

Table 5.3 Analysis data for MBC and MBN in all treatment in each day (field incubation experiment)

Time (days)	Date of analysis	slope	intercept	R^2
1 8	01 May 2012	323	8	0.999
15 22 31 46	25 May 2012	370	3	0.999
61 77 107	06 June 2012	208	15	0.999

(A fresh setting calibration was made for each day)

Figure 5.5. Soil samples taken from both sites (Day 31, 24)

Figure 5.6. Soil samples taken at day 77 from both sites

Validation of the Results

The validation of the results was performed as described in chapter 4 and the recoveries (%) of the spiked samples compared with the un-spiked samples were on average between 99 – 105 % (Table 5.4).

Table 5.4 Calculation of NO_3-N recoveries using Flow injection analysis (cadmium reduction method)

		Concentrations (mg NO_3-N/ L) measured		
Incubation period (days)	Treatments	Un-spiked sample*	Spiked sample*	Recoveries (%)
1	Unamended control plot	0.85 ± 0.07	0.92 ± 0.01	104
8	Unamended control plot	0.73 ± 0.01	0.80 ± 0.01	105
15	Unamended control plot	0.45 ± 0.05	0.52 ± 0.05	102
22	Unamended control plot	0.64 ± 0.01	0.70 ± 0.04	100
31	Unamended control plot	0.45 ± 0.01	0.52 ± 0.01	101
46	Unamended control plot	0.52 ± 0.03	0.63 ± 0.01	100
61	Unamended control plot	0.43 ± 0.015	0.49 ± 0.49	99
77	Unamended control plot	0.40 ± 0.03	0.55 ± 0.01	100
107	Unamended control plot	1.34 ± 0.02	1.40 ± 0.02	102

1 M KCl extraction solutions were spiked with 1 mL of a 1.6 mg NO_3-N /L standards in a 25 mL volumetric flask (0.064 mg/L NO_3-N).

* Values indicate mean ± sd of triplicate measurements for each treatments

The recoveries (%) for MBN measurements of the spiked samples compared with the un-spiked samples were on average between 88 – 103 % for soil samples (Table 5.5).

Table 5.5 Calculation of MBN recoveries using Flow injection analyser (cadmium reduction method)

		Concentrations (mg NO_3-N/ L) measured		
Incubation period (days)	Treatments	Un-spiked sample*	Spiked sample*	Recoveries (%)
1	Unamended control plot	0.26 ± 0.02	1.26 ± 0.01	99
8	Unamended control plot	0.68 ± 0.02	1.56 ± 0.02	88
15	Unamended control plot	0.38 ± 0.01	1.33 ± 0.01	95
22	Unamended control plot	0.29 ± 0.01	1.31 ± 0.03	102
31	Unamended control plot	0.64 ± 0.04	1.55 ± .03	91
46	Unamended control plot	0.38 ± 0.01	1.31 ± 0.01	93
61	Unamended control plot	0.44 ± 0.004	1.47 ± 0.01	103
77	Unamended control plot	0.59 ± 0.01	1.54 ± 0.01	94
107	Unamended control plot	0.31 ± 0.02	1.27 ± 0.04	96

Soil extracts (0.5 M K_2SO_4) were spiked with 5 mL of the 20mg /L nicotinic acid in 50 mL centrifuge tubes (4 mg L^{-1}).

* Values indicate mean ± sd of triplicate measurements for each treatments.

5.3 Mineralisation of N and N recoveries

The N recoveries were calculated at different time's interval between days 1 – 107 as shown in chapter 4 (Section 4.2.5). The residual mineralised fraction as a % of organic N added in the two biosolids was calculated using the same formula described in Chapter 4 (Smith and Durham, 2002).

Total N sources added from two different biosolids and fertiliser types are presented in Table 5.6. One application rate was used for two biosolids which was 510 kg N ha^{-1} and 250 kg N ha^{-1} for two fertiliser.

Table 5.6 Initial N added from two biosolids and fertiliser types amended two soil types

Biosolids and fertiliser	Total N added (kg N ha^{-1})	Mineral N added (mg)	Organic N added (mg)
ANDB	510	20065.5	2893343.5
ADB	510	38784.24	309395.5
Urea	250	-	99820
NH_4Cl	250	100100	-

5.4 Results and Discussion

5.4.1 Meteorological Data

Rainfall data for MRWP were obtained from Melton meteorology station number 087039, and rainfall data for LA were sourced from Mount Rothwell meteorology station number 087048. Monthly rainfall recorded at MRWP and LA site ranged between 33 and 49 mm during the growth period (Figure 5.7). There was no significant difference between the two sites (P-value = 0.904).

Temperature data for the two sites were obtained from Melbourne airport station number 086282 for MRWP and Avalon airport meteorology station number 087113 for LA. The average temperature from May to October was between 16.6 and 19.2 0C and between 17.1 and 19.8 °C at MRWP and LA, respectively (Figure 5.7). There was no significant difference between sites for the recorded temperature (P-value = 0.510).

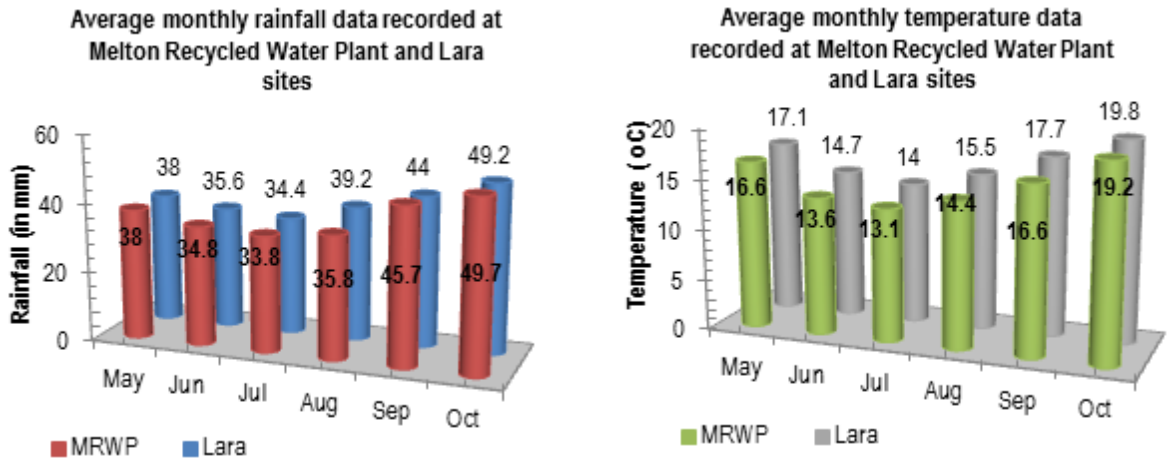

Figure 5.7. Mean monthly rainfall and temperature data recorded at Melton Recycled Water Plant (Surbiton Park) and Lara sites. Rainfall and temperature data were obtained from Bureau of Meteorology Climate Data Services, (http://www.bom.gov.au/climate/data-services/).

Soil temperature has a significant effect on the decomposition rate of biosolids and the N dynamics in the biosolids-amended soil (Barbarika et al., 1985, Pain et al., 1986, Gilmour et al., 2000, Sleutel et al., 2008, Chen et al., 2009, Wang et al., 2012). The availability of organic N reaches a maximum when the soil temperature is

between 30 – 35°C (86 – 95°F) (Deenik, 2006), and it is limited when soil temperatures are close to freezing, due inactivation of the soil microorganisms. In the experiment at MRWP and LA, the recorded soil temperatures were below the optimum values (Figure 5.8); however, there was no significant difference observed between the two sites (P-value = 0.342).

The amount of organic N mineralised in biosolids-amended soil is affected by moisture content either through rainfall or irrigation. In dry conditions, N mineralisation is low due to the activity of soil microorganisms being limited by water availability (Stanford and Epstein, 1974, Deenik, 2006). In saturated soils, the activity of microorganisms is limited by lack of oxygen. However, the effect of soil moisture on N mineralisation is a complex process (Deenik, 2006) and N mineralisation has been shown to occur under anaerobic conditions with an optimal soil moisture of 40 – 70 % of field capacity (Gilmour et al., 2000, Pierzynski and Gehl, 2005). In the current study, soil moisture data measured at the MRWP were significantly different from the LA site. The clay loam soil at The MRWP site has higher WHC (Chapter 4, Table 4.1.) which is reflected in the higher soil moisture content as shown in Figure 5.8.

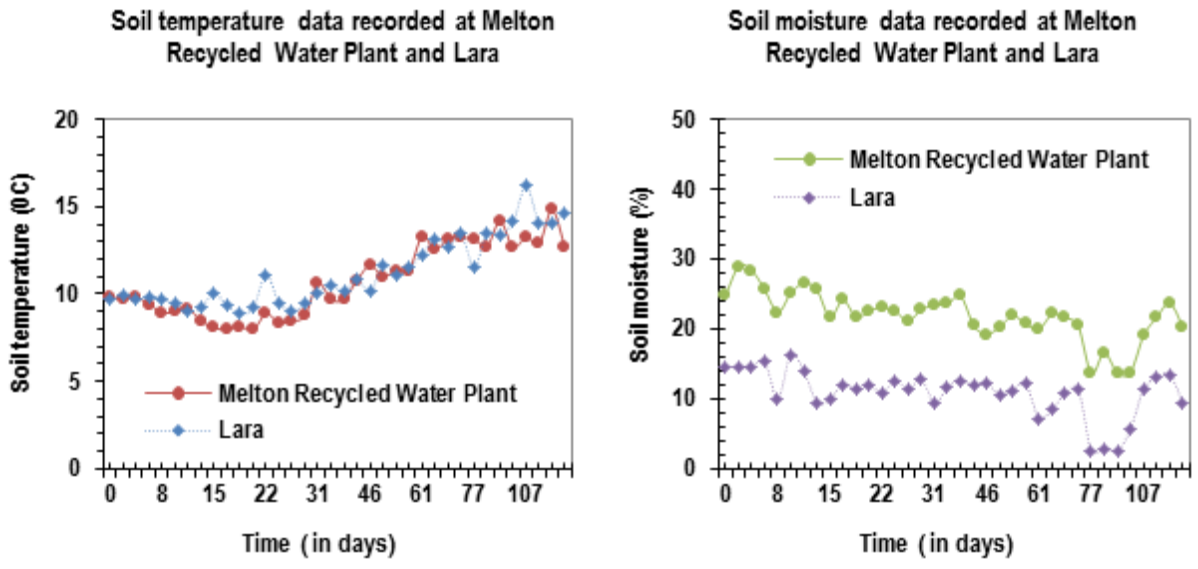

Figure 5.8. Soil temperature and moisture recorded at daily intervals during the experimental period at MRWP and LA

5.4.2 Mineral N dynamics under field conditions

Addition of mineral and organic-N can affect N dynamics in soil ecosystems. Most of the biosolids-N is in the organic form (Pu et al., 2008), and in all cases, organic N can only be available to crops when it is mineralised to NH_4-N and NO_3-N by soil microorganisms. However, anaerobically digested biosolids can contain significant proportions of total N in the form of NH_4-N (Pu et al., 2008), which can be vulnerable to loss through NH_3-N volatilisation during, and immediately after application and incorporation.

Transformations of organic-N contained in biosolids, particularly mineralisation of the large organic N fraction, need to be estimated for better understanding of N management strategies to increase crop production and minimise the potential of the movement through leaching and runoff. However, transformations of the applied N under field conditions largely depend on the composition of such applied materials, environmental conditions (such as rainfall and temperature), and the types of the soils to which the biosolids are applied, as well as the agricultural production systems. The described mineralisation rate under field conditions ranges between 15 and 55 % of the biosolids sourced organic N during the first year after application (Binder et al., 2002, Rahman and Rashid, 2002, Adegbidi and Briggs, 2003a, Vieira et al., 2005, Mendoza et al., 2006).

The quantities of N mineralised from fertiliser and biosolids-amended the clay loam and sandy loam soil are shown in Table 5.7 and Table 5.8.

Table 5.7 Quantities of mineralisation rate of two biosolids and fertiliser types amended a clay loam soil with no vegetation of ryegrass

Time (days)	Treatment types	NO_3-N (mg kg^{-1})	NH_4-N (mg kg^{-1})	MBN (mg kg^{-1})	Mineral N recoveries (mg kg^{-1})*	Mineralised N (% TN)	Mineralised N (%TN) inorganic form	Mineralised N (%TN) (sequestered in microorganism)	Mineralised N (% organic-N)
1	Unamended	29 ± 4	21 ± 4	37 ± 1					
	ANDB	43 ± 1	120 ± 14	65 ± 1	141	41.85	34.91	6.94	38.09
	ADB	73 ± 2	35 ± 0	111 ± 2	132	39.10	25.08	14.03	30.70
	Urea	44 ± 1	123 ± 16	114 ± 1	193	175.30	89.39	85.91	72.23
	NH_4Cl	65 ± 2	108 ± 2	100 ± 0	186	168.01	96.51	71.51	-
8	Unamended	34± 2	20 ± 0	22 ± 1					
	ANDB	61 ± 3	161 ± 2	23 ± 2	169	49.97	49.77	0.20	46.74
	ADB	83 ± 3	29 ± 3	15 ± 2	51	14.96	17.46	-2.50	3.06
	Urea	62 ± 3	107 ± 7	8 ± 1	100	90.63	106.45	-15.82	90.65
	NH_4Cl	66 ± 2	98 ± 8	10 ± 1	98	88.33	100.89	-12.56	-
15	Unamended	38 ± 8	16 ± 6	13 ± 2					
	ANDB	82 ± 2	55 ± 4	7 ± 4	77	22.88	24.70	-1.82	17.74
	ADB	98 ± 2	24 ± 3	2 ± 2	56	16.50	20.63	-4.14	4.78
	Urea	64 ± 3	43 ± 9	39 ± 4	80	72.26	54.24	18.02	72.28
	NH_4Cl	59 ± 2	80 ± 42	39 ± 5	112	100.97	81.63	19.33	-
22	Unamended	43 ± 2	14 ± 0	9 ± 2					
	ANDB	92 ± 2	33 ± 1	11 ± 4	69	20.50	19.91	0.59	15.20
	ADB	108 ± 1	16 ± 2	16 ± 2	73	21.61	19.69	1.92	10.62
	Urea	87 ± 1	84 ± 9	17 ± 3	120	109.14	102.32	6.82	109.16
	NH_4Cl	73 ± 3	60 ± 5	26 ± 4	92	83.54	70.43	13.11	-

Cont. Table

Time (days)	Treatment types	NO_3-N (mg kg^{-1})	NH_4-N (mg kg^{-1})	MBN (mg kg^{-1})	Mineral N recoveries (mg kg^{-1})*	Mineralised N (% TN)	Mineralised N (%TN) inorganic form	Mineralised N (%TN) (sequestered in microorganism)	Mineralised N (% organic-N)
31	Unamended	39 ± 5	27 ± 2	35 ± 9					
	ANDB	214 ± 10	30 ± 5	24 ± 3	167	49.28	52.96	-3.69	46.07
	ADB	201 ± 3	21 ± 1	44 ± 3	166	49.21	46.87	2.34	42.31
	Urea	90 ± 4	96 ± 0	35 ± 3	120	109.18	109.50	-0.32	109.20
	NH_4Cl	94 ± 4	54 ± 6	28 ± 4	75	68.20	75.90	-7.69	-
46	Unamended	35± 6	7 ± 1	16 ± 1					
	ANDB	207 ± 8	25 ± 34	16 ± 2	190	56.32	56.35	-0.03	53.48
	ADB	200 ± 3	11± 12	21 ± 1	174	51.41	49.91	1.50	44.67
	Urea	111 ± 3	33 ± 3	7 ± 1	93	84.10	93.02	-8.92	84.11
	NH_4Cl	89 ± 1	53 ± 1	38 ± 2	122	110.60	93.29	17.31	-
61	Unamended	37 ± 2	23 ± 3	16 ± 2					
	ANDB	107 ± 1	41 ± 3	14 ± 0	85	25.19	25.86	-0.67	20.24
	ADB	94 ± 2	72 ± 6	13 ± 0	62	18.30	19.28	-0.98	6.88
	Urea	94 ± 5	35 ± 1	20 ± 0	73	66.39	62.95	3.44	66.41
	NH_4Cl	54 ± 4	50 ± 3	17 ± 1	44	40.24	39.38	0.86	-
77	Unamended	20 ± 1	42 ± 3	15 ± 1					
	ANDB	75 ± 2	12 ± 5	10 ± 1	20	5.90	7.74	-1.84	-0.39
	ADB	74 ± 1	39 ± 3	15 ± 2	52	15.34	15.19	0.15	3.50
	Urea	35 ± 1	36 ± 3	63 ± 1	60	54.27	30.15	24.13	54.29
	NH_4Cl	29 ± 1	35 ± 1	11 ± 0	-2	-1.48	-0.42	-1.06	-

Cont. Table

Time (days)	Treatment types	NO_3-N (mg kg^{-1})	NH_4-N (mg kg^{-1})	MBN (mg kg^{-1})	Mineral N recoveries (mg kg^{-1})*	Mineralised N (% TN)	Mineralised N (%TN) inorganic form	Mineralised N (%TN) (sequestered in microorganism)	Mineralised N (% organic-N)
107	Unamended	18 ± 1	35 ± 0	10 ± 1					
	ANDB	89 ± 2	23 ± 8	15 ± 1	63	18.76	17.35	1.41	13.33
	ADB	87 ± 2	50 ± 0	20 ± 1	92	27.28	24.68	2.60	17.09
	Urea	48 ± 3	34 ± 2	16 ± 1	35	31.53	27.11	4.41	31.54
	NH_4Cl	49 ± 1	19 ± 1	26 ± 0	30	27.34	17.99	9.34	-

*Mineral N recoveries (NO_3-N, NH_4-N and MBN) after subtracted from the unamended control soil

Table 5.8 Quantities of mineralisation rate of two biosolids and fertiliser types amended a sandy loam soil with no vegetation of ryegrass

Time (days)	Treatment types	NO_3-N (mg kg^{-1})	NH_4-N (mg kg^{-1})	MBN (mg kg^{-1})	Mineral N recoveries (mg kg^{-1})*	Mineralised N (% TN)	Mineralised N (%TN) inorganic form	Mineralised N (%TN) (sequestered in microorganism)	Mineralised N (% organic-N)
1	Unamended	20 ± 0	12 ± 0	27 ± 1					
	ANDB	37 ± 11	134 ± 3	42 ± 1	154	45.45	41.49	3.97	40.26
	ADB	77 ± 0	23 ± 1	32 ± 2	72	21.43	20.22	1.22	9.59
	Urea	49 ± 4	98 ± 1	16 ± 2	103	93.20	105.28	-12.08	90.13
	NH_4Cl	49 ± 3	179 ± 7	17 ± 1	185	167.19	177.51	-10.32	-
8	Unamended	25 ± 4	18 ± 1	7 ± 1					
	ANDB	63 ± 7	111 ± 7	32 ± 1	157	46.35	40.02	6.34	41.16
	ADB	59 ± 3	20 ± 6	20 ± 0	50	14.93	11.81	3.12	2.36
	Urea	52 ± 2	19 ± 7	26 ± 1	48	43.85	31.16	12.69	42.41
	NH_4Cl	48 ± 3	64 ± 35	18 ± 0	80	72.63	63.96	8.67	-
15	Unamended	26 ± 2	9 ± 2	17 ± 2					
	ANDB	54 ± 1	55 ± 8	14 ± 3	71	21.08	21.78	-0.69	15.04
	ADB	57 ± 1	17 ± 9	21 ± 3	42	12.58	11.45	1.13	-0.23
	Urea	55 ± 3	63 ± 3	62 ± 6	127	115.69	85.41	30.28	111.87
	NH_4Cl	48 ± 7	95 ± 5	19 ± 5	110	99.60	97.59	2.01	-
22	Unamended	22 ± 0	24 ± 4	8 ± 2					
	ANDB	70 ± 1	41 ± 7	17 ± 4	73	21.68	19.34	2.34	15.66
	ADB	89 ± 5	23 ± 2	11 ± 2	68	20.06	19.21	0.85	8.06
	Urea	74 ± 2	86 ± 7	18 ± 6	123	112.05	103.71	8.34	108.36
	NH_4Cl	70 ± 2	84 ± 51	19 ± 3	118	106.58	97.64	8.94	-

Cont. Table

Time (days)	Treatment types	NO_3-N (mg kg^{-1})	NH_4-N (mg kg^{-1})	MBN (mg kg^{-1})	Mineral N recoveries (mg kg^{-1})*	Mineralised N (% TN)	Mineralised N (%TN) inorganic form	Mineralised N (%TN) (sequestered in microorganism)	Mineralised N (% organic-N)
31	Unamended	23 ± 1	29 ± 4	19 ± 2					
	ANDB	111 ± 1	100 ± 6	35 ± 2	174	51.37	47.19	4.18	46.42
	ADB	181 ± 5	34 ± 0	28 ± 2	126	48.01	45.62	2.39	39.02
	Urea	104 ± 3	91 ± 15	21 ± 2	145	131.31	129.73	1.58	126.98
	NH_4Cl	60 ± 0	89 ± 19	29 ± 2	107	96.90	89.02	7.88	-
46	Unamended	21 ± 5	28 ± 4	10 ± 1					
	ANDB	88 ± 6	12 ± 2	20 ± 0	61	17.94	15.49	2.45	11.81
	ADB	144 ± 24	20 ± 9	30 ± 2	136	40.17	35.07	5.10	30.32
	Urea	99 ± 2	70 ± 11	6 ± 1	116	105.42	109.17	-3.75	101.94
	NH_4Cl	31 ± 4	40 ± 10	55 ± 7	66	59.94	35.67	24.27	-
61	Unamended	17 ± 1	31 ± 4	14 ± 0					
	ANDB	44 ± 2	41 ± 8	20 ±1	43	12.58	11.02	1.56	6.27
	ADB	44 ± 4	52 ± 9	19 ± 1	52	15.32	13.95	1.37	2.85
	Urea	49 ± 5	45 ± 32	11 ± 1	42	38.52	41.06	-2.54	37.26
	NH_4Cl	35 ± 2	34 ± 14	18 ± 0	24	21.99	18.99	3.00	-
77	Unamended	21 ± 1	33 ± 3	9 ± 0					
	ANDB	25 ± 1	56 ± 8	9 ± 1	27	8.05	8.13	-0.08	1.59
	ADB	36 ± 1	39 ± 2	7 ± 0	19	5.51	6.43	-0.92	-8.00
	Urea	30 ± 1	37 ± 1	21 ± 2	23	21.28	14.34	6.94	20.58
	NH_4Cl	11 ± 0	45 ± 47	15 ± 3	8	7.22	4.17	3.05	-

Cont. Table

Time (days)	Treatment types	NO_3-N (mg kg^{-1})	NH_4-N (mg kg^{-1})	MBN (mg kg^{-1})	Mineral N recoveries (mg kg^{-1})*	Mineralised N (% TN)	Mineralised N (%TN) inorganic form	Mineralised N (%TN) (sequestered in microorganism)	Mineralised N (% organic-N)
107	Unamended	12 ± 0	39 ± 6	7 ± 1					
	ANDB	32 ± 1	23 ± 4	26 ± 2	22	6.53	3.55	2.98	0.00
	ADB	54 ± 2	54 ± 0	15 ± 1	65	19.27	17.19	2.07	7.19
	Urea	30 ± 1	39 ± 2	7 ± 0	18	16.07	16.73	-0.67	15.55
	NH_4Cl	19 ± 1	38 ± 7	11 ± 3	10	8.78	6.40	2.38	-

*Mineral N recoveries (NO_3-N, NH_4-N and MBN) after subtracted from the unamended control soil

5.4.2.1 Mineral N dynamics of NH_4Cl in the clay loam soil

The change of mineral N (NH_4-N and NO_3-N) in NH_4Cl-applied to the clay loam soil is presented in Figure 5.9.

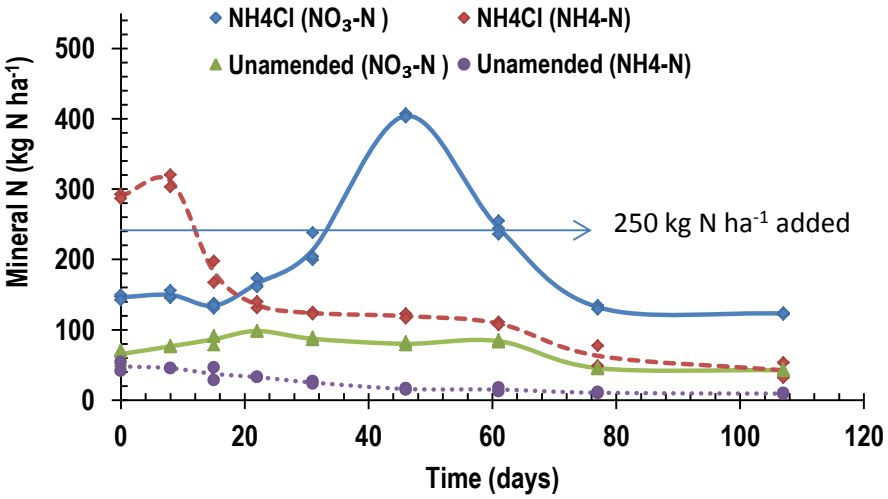

Figure 5.9. The effect of applying NH_4Cl on NO_3-N and NH_4-N dynamics on the clay loam soil (n= 3)(the connecting lines show the mean of triplicate measurements plotted against times)

Production of mineral N (NH_4-N and NO_3-N) from NH_4Cl

The NH_4-N concentration in NH_4Cl recorded in the clay loam was higher (303 kg N ha^{-1}) on day 8, after that there was a slow decline.

The highest level of NO_3-N was 404 kg N ha^{-1} on day 46, which was three times higher than the unamended control soil. The nitrification rate produced per day was 8.78 kg N ha^{-1}. The majority of added NH_4-N had been transformed to NO_3-N during this period. In a field study conducted by Rigby (2008), in silty clay soil amended with NH_4Cl, there was an increase in nitrification rate between days 0 – 34 which was equivalent to 4.44 kg N ha^{-1} day^{-1}. The nitrification rate produced in this study was double. However, NO_3-N concentration decreased after day 46, indicating movement of NO_3-N below the amended soil depth (0 – 15 cm). The nitrification process in NH_4Cl was substantially higher ($P > 0.05$) than the unamended control plot during the experiment period.

5.4.2.2 Microbial biomass N and C

Many researchers have reported organic compounds are subjected to metabolism by microorganisms within a relatively short time of being presented to the soil (Kiem and Kögel-Knabner, 2003, Lützow et al., 2006, Marschner et al., 2008).

Generally, the MBN ranged between 100 – 140 kg N ha^{-1} and 300 – 1200 kg C ha^{-1} in comparison to the clay loam soil which was in the range of 227 – 59 kg N ha^{-1} and 498 – 11 kg C ha^{-1} (Figure 5.10). These results are consistent with the lager microbial population activity in the clay loam than in the sandy loam soil and these results within normal ranges (Banerjee et al., 1997, Franco-Hernández et al., 2003, Jedidi et al., 2004, Fernandes et al., 2005).

Microbial biomass N and C in the clay loam soil amended with NH_4Cl are shown in Figure 5.10.

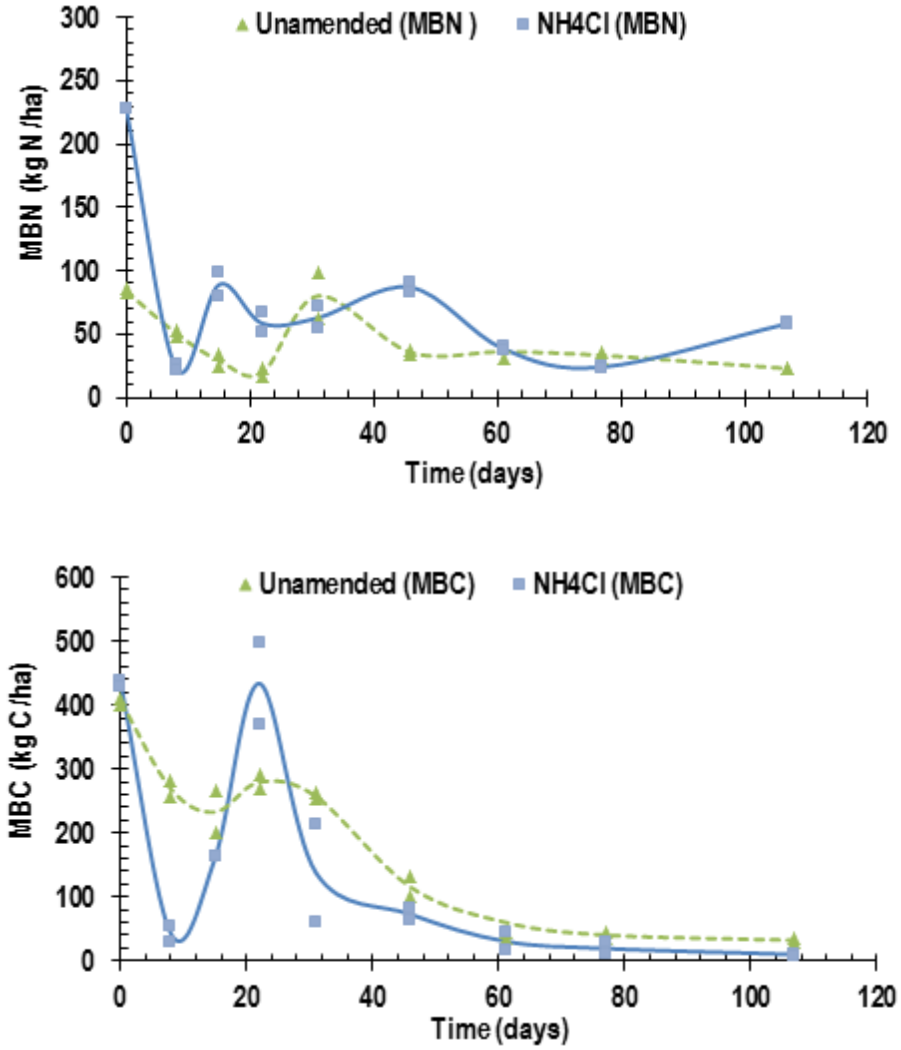

Figure 5.10. The influence of applying NH_4Cl on MBN and MBC dynamics on clay loam soil (n=2) (the connecting lines show the mean of duplicate measurements plotted against times)

In the NH_4Cl-amended clay loam soil, the level of MBN was higher at beginning of the experiment and decreased to 23 kg N ha^{-1} on day 8 after that, it fluctuated. There was a significantly ($P < 0.05$) higher MBN concentration found in NH_4Cl treatment than the unamended control plots.

When NH_4Cl was applied to the clay loam soil, the MBC level initially decreased between days 0 – 8 and then unexpectedly increased to 433 kg C ha^{-1} on day 22. However, after this period the levels of MBC decreased.

Microbial biomass ratio

Microbial biomass C to N ratio in NH_4Cl over 107 days is shown in Figure 5.11

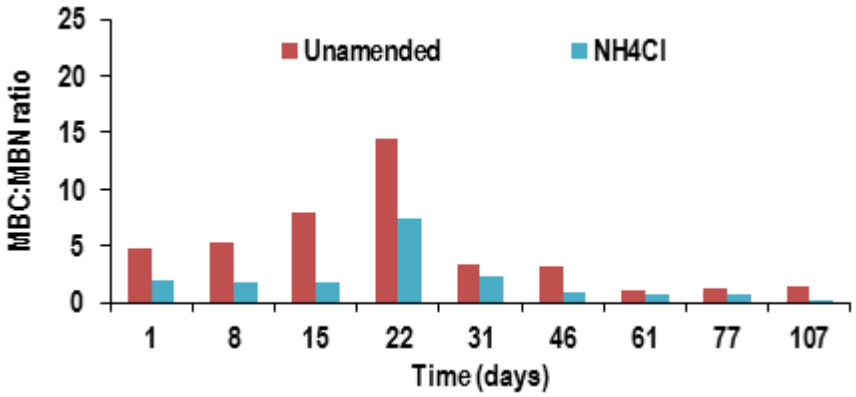

Figure 5.11. The ratio of microbial biomass C to N (mean, n = 3) in the NH_4Cl-treated clay loam soil

In the unamended clay loam soil, the ratio was slightly increased and extents to a maximum value on day 22. After this point, it deceased over 107 days. Similar observation were observed in NH_4Cl amended the clay loam soil. However, comparing with NH_4Cl amendment under laboratory incubation, the maximum ratio of microbial biomass was 57 on day 1.

Comparing the laboratory incubation and field incubation experiment, the ratio of biomass C to N in the clay loam soil amended with NH_4Cl shows that there was an initial increase following the application rate under laboratory conditions. Under the field condition, there was an increase of the ratio C: N biomass that reached a maximum on day 22. The ratio of MBC: MBN biomass was greater in the laboratory incubation than field incubation.

5.4.2.3 Mineral N dynamics of NH_4Cl in the sandy loam soil

The rate of ammonification (NH_4-N) and nitrification (NO_3-N) processes of organic N in NH_4Cl-applied to the sandy loam soil soils is presented in Figure 5.12

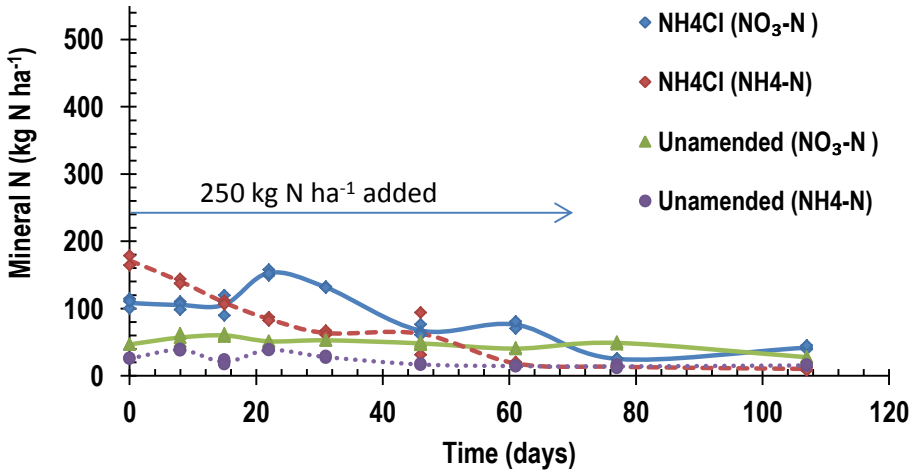

Figure 5.12. The effect of applying NH_4Cl on NO_3-N and NH_4-N dynamics on the sandy loam soil (n=3) (the connecting lines show the mean of triplicate measurements plotted against times)

Production of mineral N (NH_4-N and NO_3-N) from NH_4Cl

The maximum concentration of NH_4-N in NH_4Cl-treated sandy loam soil was recorded on day 1 was 164 kg N ha^{-1} after which there was a gradual decline. Comparing with the clay loam soil receiving NH_4Cl, the concentration of NH_4-N observed was greater than the amount of ammonification found in the sandy loam soil.

The ammonification rate in NH_4Cl-amended sandy loam soil was significantly ($P < 0.001$) higher than the unamended control soil throughout the trial period.

On the other hand, in the NH_4Cl amended sandy loam soil, the NO_3-N was highest (148 kg N ha^{-1}) by day 22, a level that was about three times greater than the unamended control plot. The nitrification process was lower in sandy loam than the clay loam soil. This is consistent with the observation of ammonification and nitrification process observed in two soil types amended with NH_4Cl under laboratory incubation condition.

As a result of t-test using ANOVA, there was significant difference ($P < 0.05$) in the nitrification process between NH_4Cl and unamended control plot.

5.4.2.4 Microbial biomass N and C

The concentration of MBN and MBC in NH_4Cl-amended the sandy loam soil over 107 days is presented in Figure 5.13.

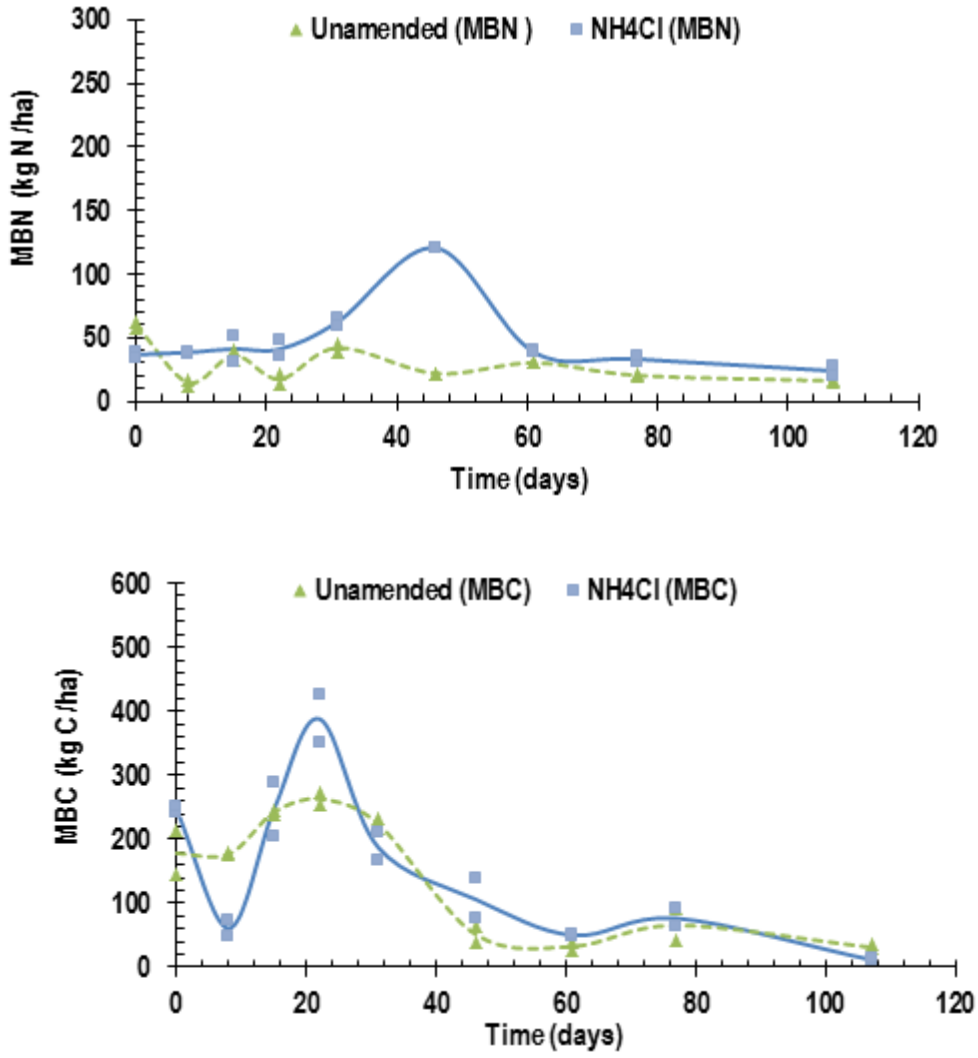

Figure 5.13. The influence of applying NH_4Cl on MBN and MBC dynamics on sandy loam soil (n=2) (the connecting lines show the mean of duplicate measurements plotted against times)

In the NH_4Cl-treated plots, the MBN concentration was relatively constant between days 1 – 31. It then reached a maximum at 120 kg N ha^{-1} on day 46, where it was 4 times greater than unamended control plot and then decreased. Microbial growth changes may be relative to changes in the soil conditions. Soil temperature

increased in both soil types but the soil moisture in the clay loam soil was in a better condition compared to sandy loam soil during the course of the experiment.

The MBC concentration in the NH_4Cl-amended sandy loam soil reached a maximum value of 388 kg C ha^{-1} on day 22 and then decreased over a the remaining time.

Microbial biomass ratio

Microbial biomass C to N ratio over 107 days is shown in Figure 5.14. The ratio of MBC: MBN in NH_4Cl showed a similar behaviour in the clay loam soil as presented in Figure 5.11

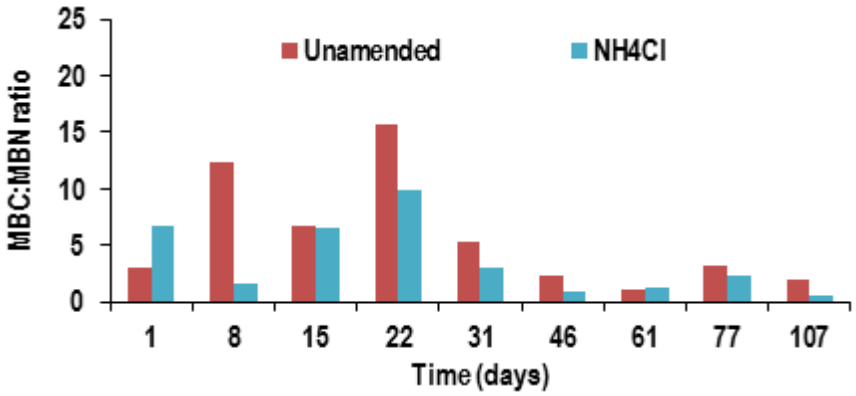

Figure 5.14. The ratio of microbial biomass C to N (mean, n = 3) in the NH_4Cl-treated sandy loam soil

The ratio of MBC: MBN increased, which is opposite to the behaviour in the incubation experiment. N leaching in the field may explain theses differences. A similar pattern was observed for the clay loam soil which it may be due to leaching process.

Comparison between two soil types amended with NH_4Cl

The amount of mineral N was approximately 168 % of total N added in the clay loam soil as shown in Figure 5.15. The negative values on day 8 and 31 in the clay loam soil showed there was movement of NO_3-N below the soil depth (0 -15 cm).

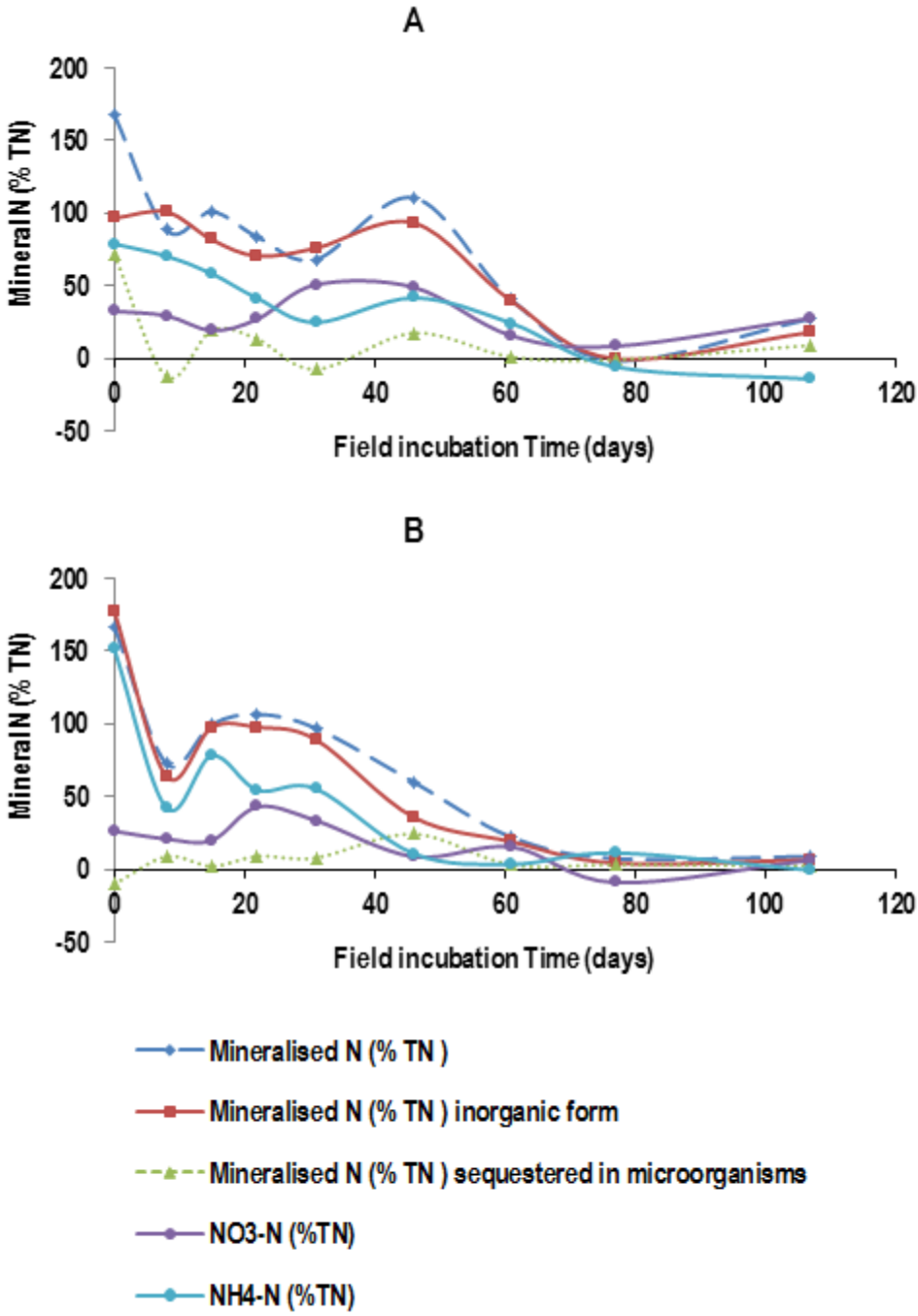

Figure 5.15. Mineral N (%TN added) from NH_4Cl-applied to the clay loam (A) and sandy loam soil (B)

The amount of mineral N, MBN as percentage of total N added from NH_4Cl and the maximum fraction of the organic-N mineral under different conditions is shown in Table 5.9. In general, the mineral N observed in the clay loam soil was greater than sandy loam soil as observed in the laboratory experiment. However, the availability of N from NH_4Cl was greater than the amount of total N added, which may mean

there was an error in the quantitiy mass of applied. However, it was also assumed that the amendments were mixed to a depth of 15 cm.

Table 5.9 Mineral N from NH_4Cl-amended soils in the laboratory and field incubation experiments

NH_4Cl		Day of maximum mineralised N	% TN_{added} NH_4-N	% TN_{added} NO_3-N	% TN_{added} MBN	Mineralised N (% TN)
Clay loam soil	Lab	1	83 ± 1	1± 1	1 ± 0.1	85 ± 2
	Field	1	79 ± 1	32 ± 2	71 ± 10	168 ± 9
Sandy loam soil	Lab	1	80 ± 2	-1 ± 1	1 ± 1	78 ± 3
	Field	1	151 ± 5	27 ± 1	-10 ± 4	167 ± 9

5.4.2.5 Mineral N dynamics of urea in the clay loam soil

The change of the NH_4-N and NO_3-N concentrations in urea-applied to the clay loam soil soils is presented in Figure 5.16.

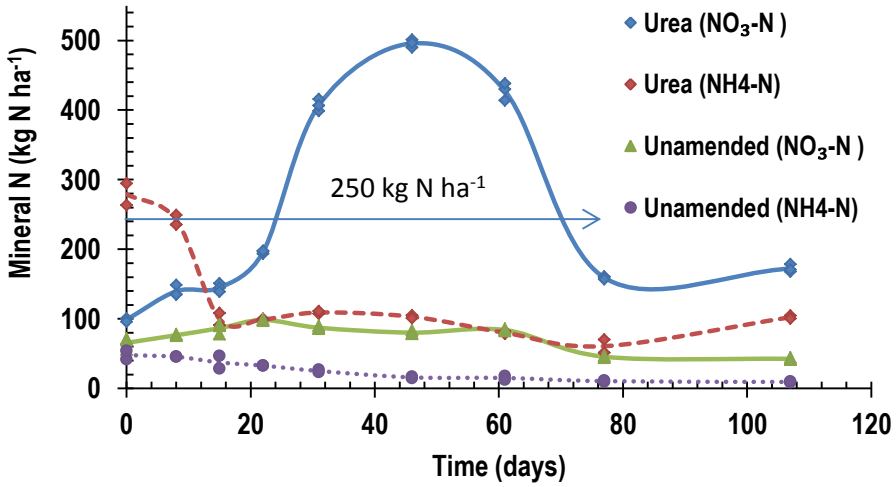

Figure 5.16. The effect of applying urea on NO_3-N and NH_4-N dynamics on the clay loam soil (n=3) (the connecting lines show the mean of triplicate measurements plotted against times)

Production of mineral N (NH_4-N and NO_3-N) from urea

In the clay loam soil treated with urea, there was reduction in NH_4-N between days 1 – 15 as a result of the transformation of NH_4-N transformed to NO_2-N and then to NO_3-N. The concentration of NH_4-N between days 15 – 107 slowly declined.

The concentration of NO_3-N in urea amended in a clay loam soil increased reaching a maximum at 489 kg N ha^{-1} by day 46. The difference here is 384 kg N ha^{-1} which is three times greater than unamended control plot. The nitrification rate was 11 kg N ha^{-1} day^{-1}. By day 46 onwards the levels of NO_3-N decreased. The decrease may be a result of movement of NO_3-N below 15 cm soil depth or it may have been sequestered by microorganisms or converted to other forms.

5.4.2.6 Microbial biomass N and C

The concentration of MBN and MBC in urea-amended the clay loam soil over 107 days is presented in Figure 5.17.

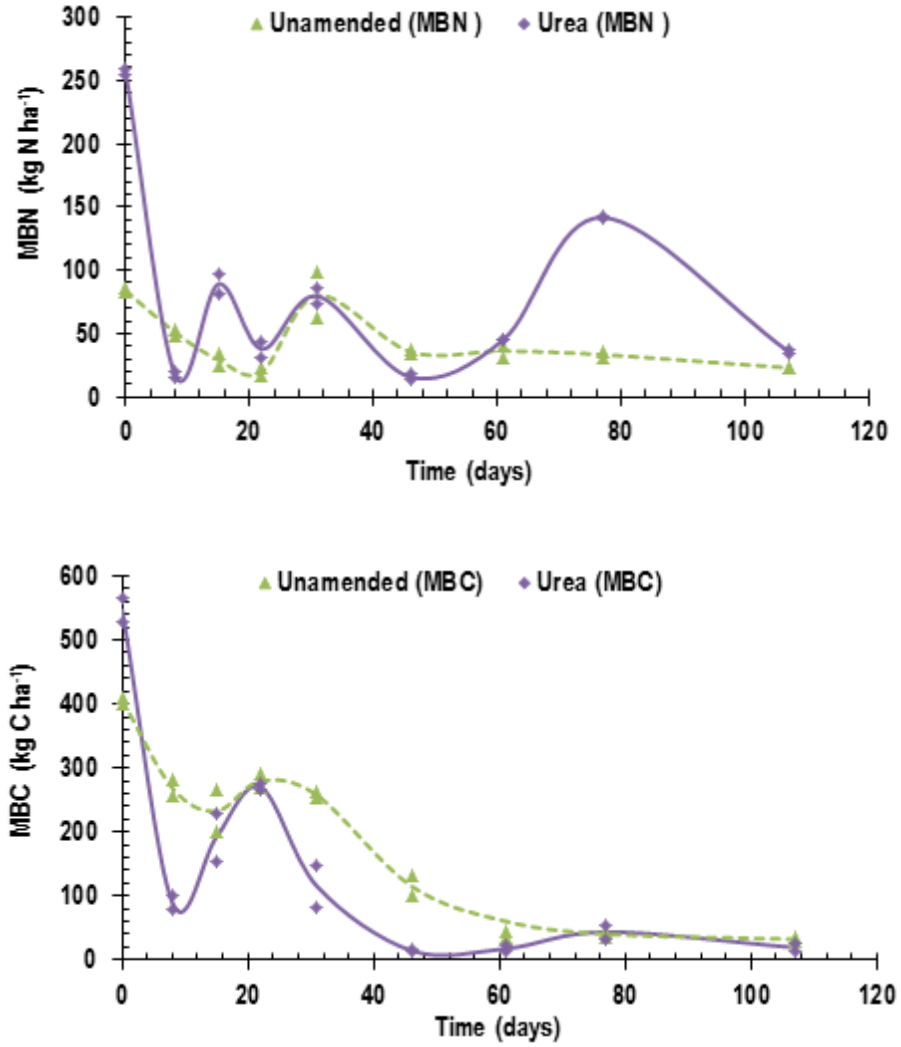

Figure 5.17. The influence of applying urea on MBN and MBC dynamics on clay loam soil (n=2) (the connecting lines show the mean of duplicate measurements plotted against times)

There was an increase in MBN concentration observed from urea amended clay loam soil on day 1 which dropped to 17 kg N ha^{-1} by day 8. MBN concentration fluctuated between days 8 – 61. However, there was an increase of MBN concentration (77 kg N ha^{-1}) which may be due to increasing the soil temperature and decreasing moisture content as shown in Figure 5.8. The concentration of MBC detected in clay loam soil amended with urea was highest initially but within 8 days sharply decreased and then increase up to 265 kg C ha^{-1} which is similar to the unamended control plot on day 22. It then decreased over the remaining period.

Microbial biomass ratio

The change of microbial biomass C to N ratio in urea-amended the clay loam soil over 107 days is shown in Figure 5.18. The ratio of MBC: MBN in urea was higher on day 22. This is consistent with NH_4Cl-applied on the same soil (Figure 5.11).

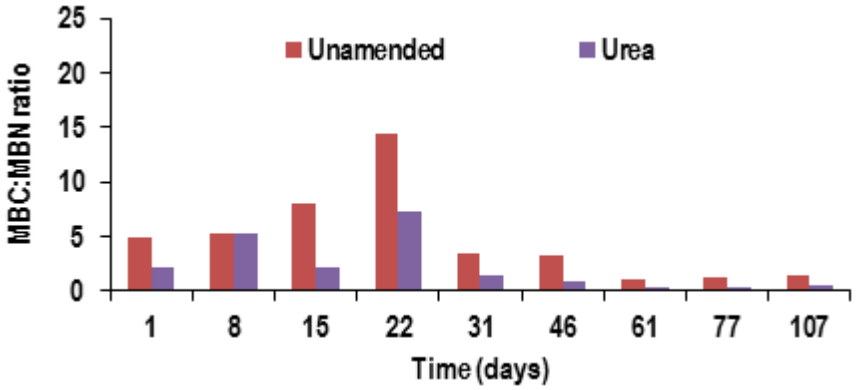

Figure 5.18. The ratio of microbial biomass C to N (mean, n = 3) in the urea-amended clay loam soil

Comparing the ratio of microbial biomass C to N in urea-amended the clay loam under different conditions, there was an increase of the ratio at the beginning on day 1, again this is opposite to the behaviour in laboratory experiment. It is consistent with other fertiliser used in this study.

5.4.2.7 Mineral N dynamics of urea in the sandy loam soil

The change of ammonification (NH_4-N) and nitrification (NO_3-N) processes of organic-N in urea-applied to the sandy loam soil is shown in Figure 5.19.

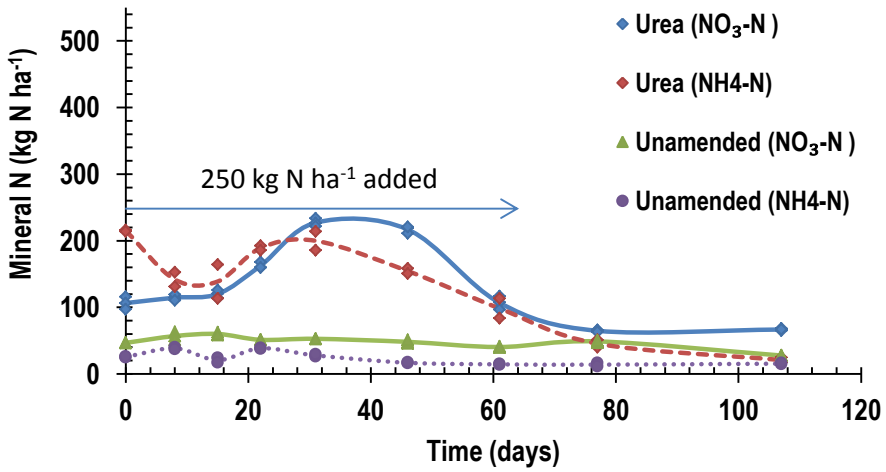

Figure 5.19. The effect of applying urea on NO_3-N and NH_4-N dynamics on the sandy loam soil (n=3) (the connecting lines show the mean of triplicate measurements plotted against times)

Production of mineral N (NH_4-N and NO_3-N) from urea

In sandy loam soil treated with urea, NH_4-N concentrations decreased between days 1 – 8 which indicates that most of NH_4-N was transformed to NO_3-N through the nitrification process. There was a slight increase until day 31 after which NH_4-N decreased. The concentration of NH_4-N in unamended sandy loam soil was relatively constant and below the urea treatment over 107 days.

In the sandy loam soil receiving urea, the level of NO_3-N reached a maximum of 216 kg N ha^{-1} on day 46. On the same day in unamended sandy loam soil, NO_3-N was 48 kg N ha^{-1}. The amount of NO_3-N had been mineralised from urea was approximately three times greater than the unamended sandy loam soil Comparing between two soil types, there was great nitrification process in the clay loam than sandy loam soil. However, the amount of NO_3-N released from urea-amended the clay loam soil was higher than sandy loam soil which is relevant to the results obtained from the laboratory incubation experiment.

5.4.2.8 Microbial biomass N and C

The concentration of MBN and MBC in urea-amended the sandy loam soil over 107 days is presented in Figure 5.20

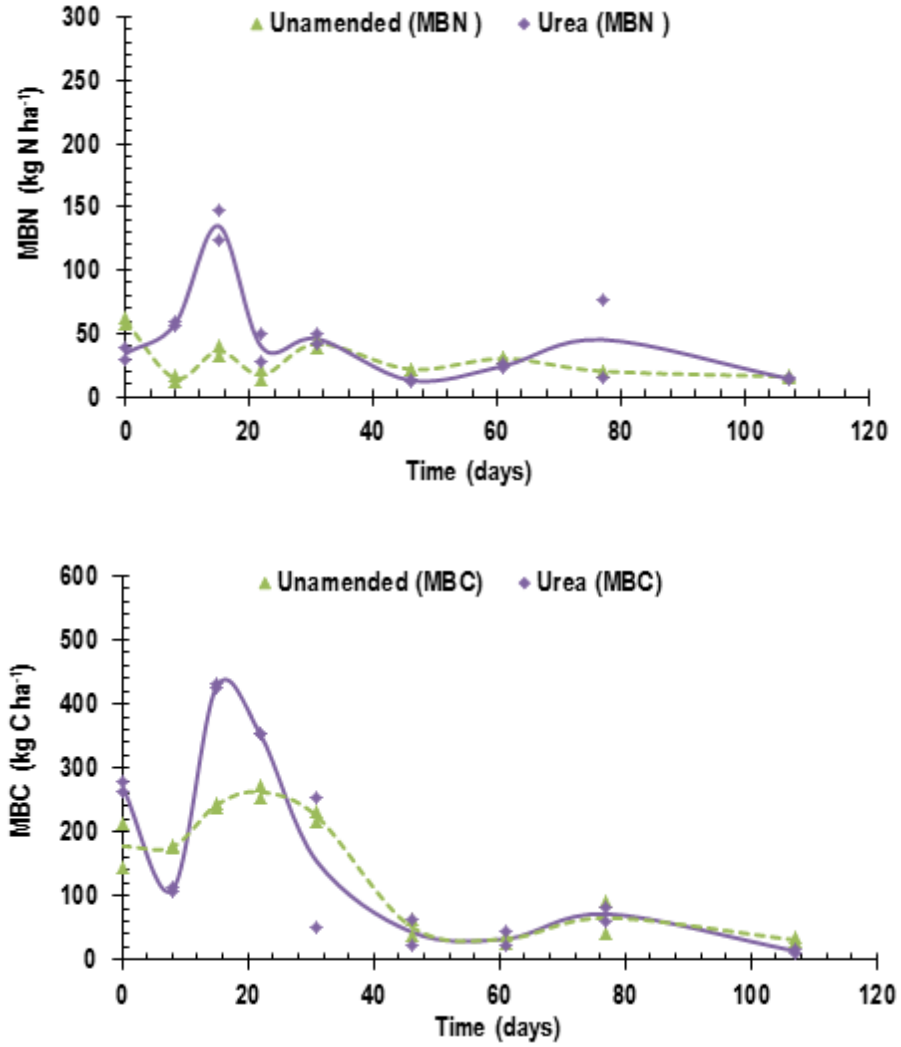

Figure 5.20. The influence of applying urea on MBN and MBC dynamics on sandy loam soil (n=2) (the connecting lines show the mean of duplicate measurements plotted against times)

In the sandy loam soil treated with urea, the concentrations of MBN increased to 135 kg N ha^{-1} between days 1 – 15 and then slightly decreased over the remaining time.

The MBC concentration decreased from 272 kg C ha^{-1} to 109 kg C ha^{-1} within 8 days and increased to 427 kg C ha^{-1} by day 15 after that MBC decreased.

Microbial biomass ratio

Microbial biomass C to N ratio in urea-amended the sandy loam soil over 107 days is shown in Figure 5.21. The ratio of MBC: MBN was highest on day 22; this is consistent with NH_4Cl applied to the same soil (Figure 5.14). However, the biomass ratio decreased after day 22.

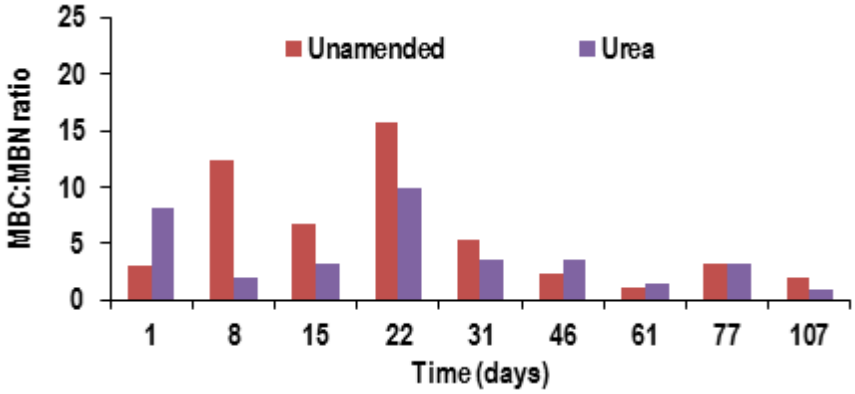

Figure 5.21. The ratio of microbial biomass C to N (mean, n = 3) in the urea-treated sandy loam soil

Comparison between two soil types amended with Urea

In the field site with the clay loam soil, the amount of N mineralised was approximately 175 % the day after the application and 131 % in the sandy loam soil on day 31.(Figure 5.22) The amount of mineral N observed in urea-treated the clay loam soil was approximately 175 % of total N added after following the application rate. In the sandy loam soil receiving urea, the highest amount of mineral N was 131 % of total N added was on day 31. The delay in mineralisation and nitrification may be due to lower fertility and soil texture.

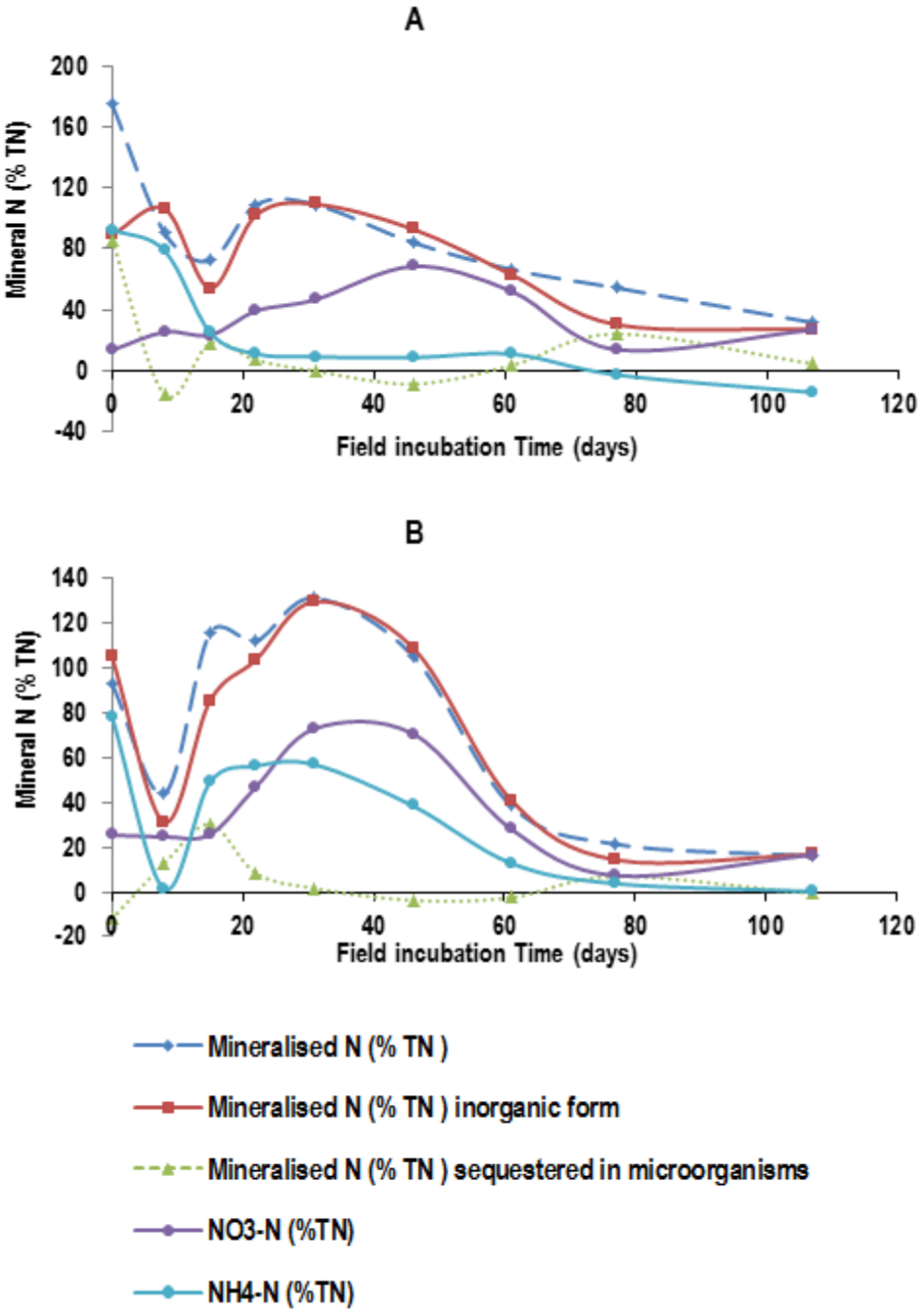

Figure 5.22. Mineral N (%TN added) from urea-applied to the clay loam (A)and sandy loam soil (B)

The ammonification, nitrification process, MBN (%TN), total N mineralised as % TN added and the highest proportion of the organic-N mineralised in the case of urea-amended two soil types are presented in Table 5.10. In the clay loam soil, nitrification rates were greater under laboratory incubation conditions than under the field conditions. However, the availability of N from urea-amended the clay loam soil

under both conditions showed that there was more N available than total N added. However, it was not as high as in the laboratory experiment.

Table 5.10 Mineral N from urea-amended soils in the laboratory and field incubation experiments

Urea		Day of maximum mineralised N	% TN_{added} NH_4-N	% TN_{added} NO_3-N	% TN_{added} MBN	Mineralised N (% TN)
Clay loam soil	Lab	42	54 ± 2	148 ± 2	18 ± 1	225 ± 19
	Field	1	92 ± 3	13 ± 1	85 ± 11	175 ± 7
Sandy loam soil	Lab	8	94 ± 2	17 ± 1	1 ± 1	111 ± 14
	Field	31	56 ± 2	73 ± 3	2 ± 1	131 ± 11

5.4.2.9 Mineral N dynamics of ANDB in the clay loam soil

The rate of ammonification (NH_4-N) and nitrification (NO_3-N) processes of organic-N in ANDB-applied to the clay loam soil is shown in Figure 5.23.

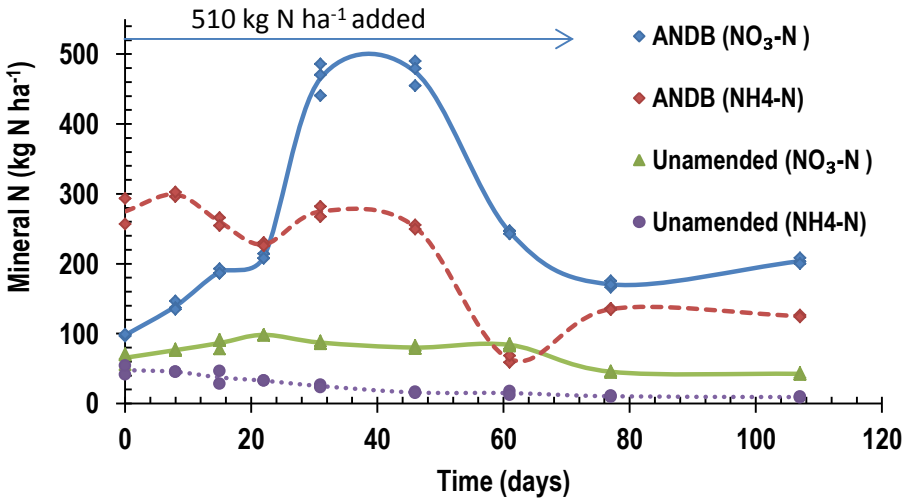

Figure 5.23. The effect of applying ANDB on NO_3-N and NH_4-N dynamics on the clay loam soil (n=3) (the connecting lines show the mean of triplicate measurements plotted against time)

Production of mineral N (NH_4-N and NO_3-N) from ANDB

The concentration of NH_4-N in the clay loam soil amended with ANDB between days 1 – 22 decreased from 293 to 214 kg N ha^{-1}, which was 12 times greater than the NH_4-N values recorded in unamended a clay loam soil. However, after day 22 onwards the concentration of NH_4-N decreased until the end of the experiment period.

The concentration of NO_3-N in the clay loam soil amended with ANDB increased and reached a maximum of 474 kg N ha^{-1} on day 46. The production of NO_3-N in ANDB was four times greater than NO_3-N produced in the unamended control plots and the nitrification rate was 11 kg N $ha^{-1}day^{-1}$. However, the level of NO_3-N decreased after day 46. The NO_3-N concentration observed in the unamended control plot was relatively consistent over the 107 days.

5.4.2.10 Microbial biomass N and C

The change of the MBN and MBC concentrations in ANDB-amended the clay loam soil over 107 days are presented in Figure 5.24.

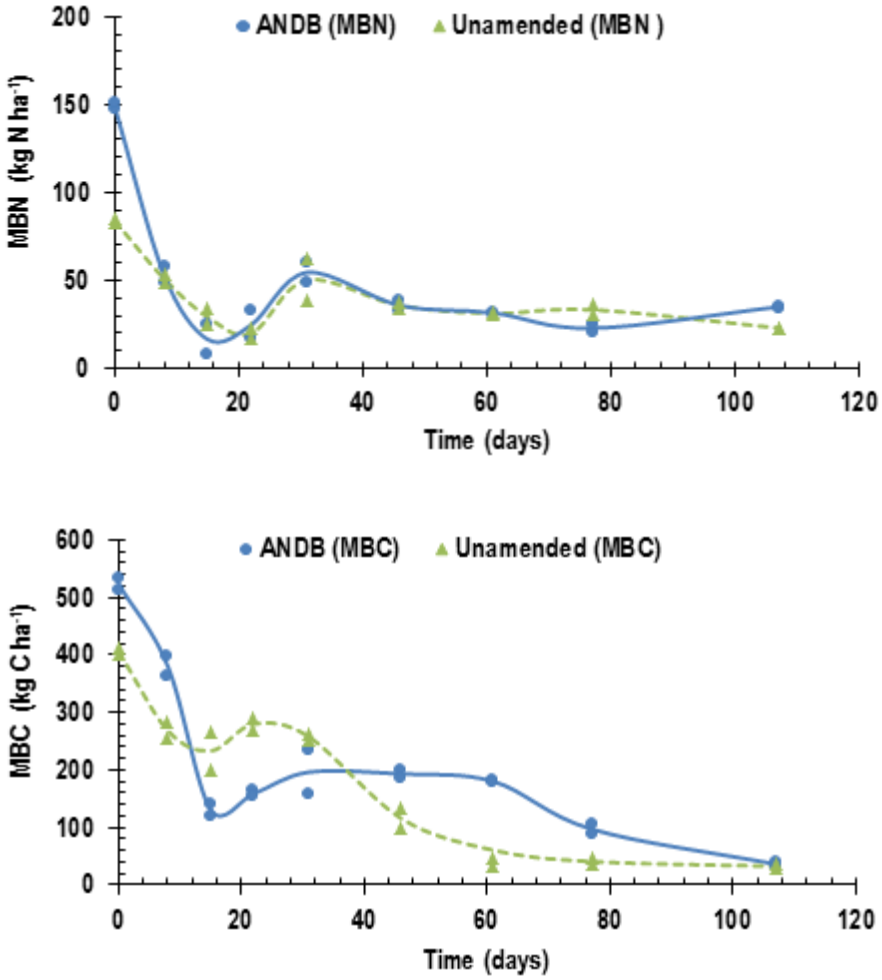

Figure 5.24. The influence of applying ANDB on MBN and MBC dynamics on clay loam soil (n=2) (the connecting lines show the mean of duplicate measurements plotted against times)

In the clay loam soil amended with ANDB without vegetation, the greatest concentration of MBN was 147 kg N ha^{-1} after adding ANDB to the soil (day 1) and dropped down to 16 kg N ha^{-1} by day 15 and then increase again to 54 kg N ha^{-1} by day 31 and then decreased.

The highest concentration of MBC observed from ANDB was 513 kg C ha^{-1} on day 1 following the application rate. However, the concentration of MBC decreased to 120 kg C ha^{-1} by day 15 and then slightly declined.

Microbial biomass ratio

The ratio of MBC to MBN in ANDB-amended the clay loam soil over 107 days is shown in Figure 5.25. The ratio of MBC: MBN was highest on day 15 and then decreased.

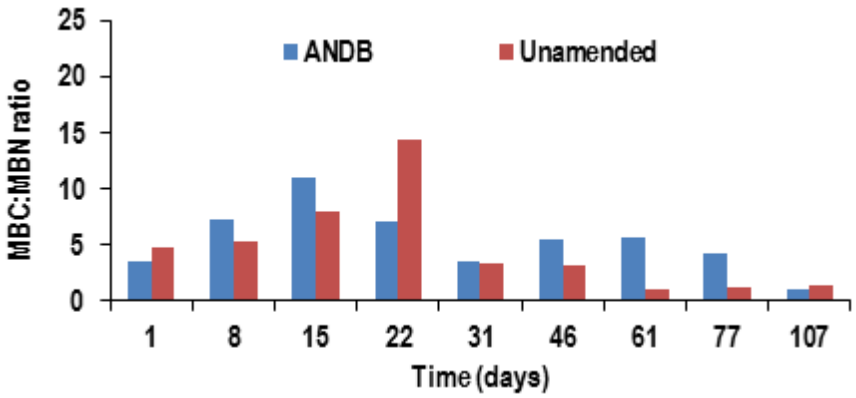

Figure 5.25. The ratio of microbial biomass C to N (mean, n = 3) in the ANDB-treated clay loam soil

Similar behaviour was observed in the ratio of microbial biomass C to N in both fertilisers.

5.4.2.11 Mineral N dynamics of ANDB in the sandy loam soil

The concentrations levels of NH_4-N and NO_3-N of organic-N in ANDB-applied to the sandy loam soil are shown in Figure 5.26.

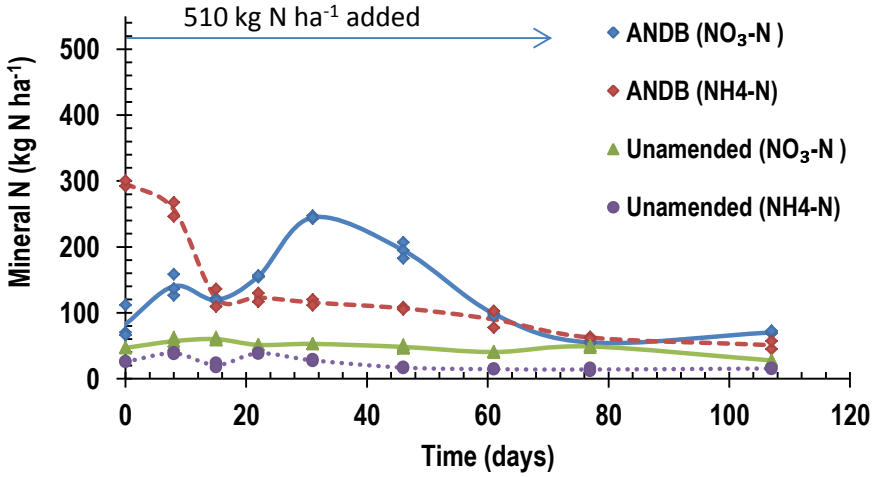

Figure 5.26. The effect of applying ANDB on NO_3-N and NH_4-N dynamics on the sandy loam soil (n=3) (the connecting lines show the mean of triplicate measurements plotted against times)

Production of mineral N (NH_4-N and NO_3-N) from ANDB

The levels of NH_4-N in sandy loam soil amended with ANDB during days 1 were the highest with a maximum of 292 kg N ha^{-1}, but between days 8 – 15 NH_4-N level decreased to 135 kg N ha^{-1}. From days 15 – 107 ammonification decreased with a corresponding increase in NO_3-N.

The level of NO_3-N in ANDB treated sandy loam soil gradually increased to 245 kg N ha^{-1} which was equal to 40 kg N ha^{-1} by day 31. The production of NO_3-N produced from ANDB was three times more than the unamended clay loam soil. At this point, the rate of nitrification was 8 kg N ha^{-1} day^{-1}. However, by day 31 to day 107, the change of NO_3-N decreased which indicates a loss of N. The changes of the concentration of NO_3-N observed from the unamended control plots were relatively consistent over 107 days.

5.4.2.12 Microbial biomass N and C

The concentrations of MBN and MBC in ANDB-amended the sandy loam soil over 107 days are presented in Figure 5.27.

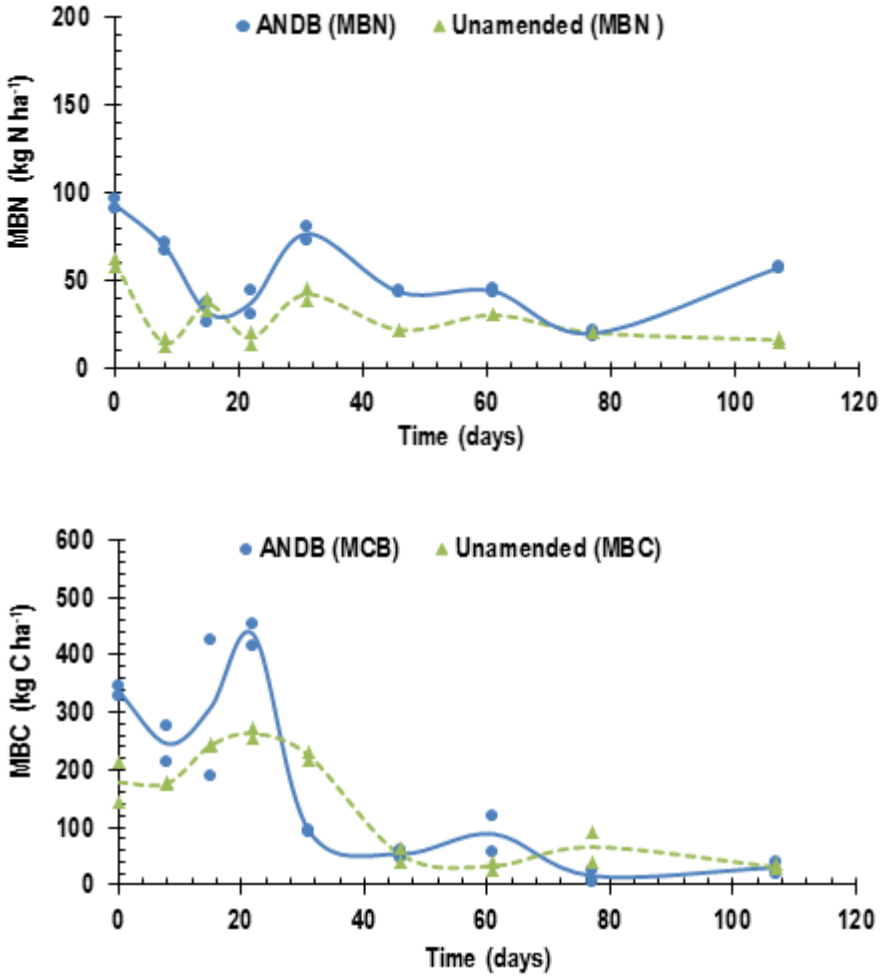

Figure 5.27. The influence of applying ANDB on MBN and MBC dynamics on sandy loam soil (n=2) (the connecting lines show the mean of duplicate measurements plotted against times)

MBN levels in ANDB-amended sandy loam soils decreased between days 1 – 22 and then reached a maximum level at 76 kg N ha^{-1} by day 31. After that, the levels of MBN fluctuated.

On the other hand, the MBC concentration in the ANDB-treated sandy loam soil increased to 453 kg C ha^{-1} on day 22. After that, the MBC concentration dropped. The statistical analysis shows that there was no significant different ($P > 0.05$) in MBC between the ANDB-amended the clay loam and sandy soil and the unamended control soil.

Microbial biomass ratio

Microbial biomass C to N ratio in ANDB-amended the sandy loam soil over 107 days is shown in Figure 5.28. The ratio of MBC: MBN was highest on day 22 and then decreased.

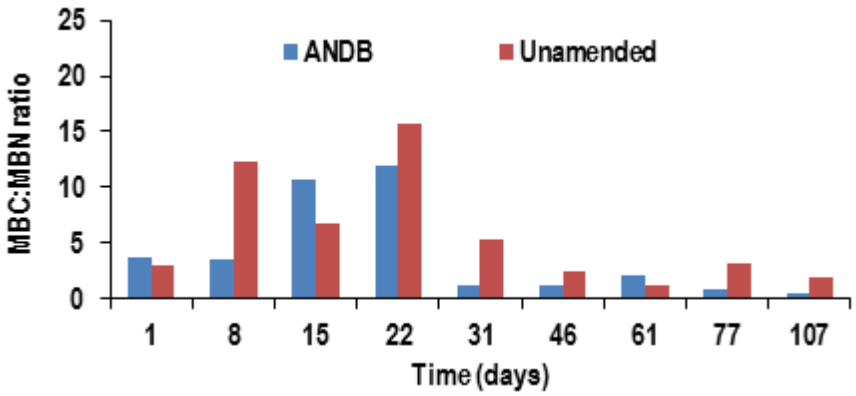

Figure 5.28. The ratio of microbial biomass C to N (mean, n = 3) in the ANDB-treated sandy loam soil

The ratio observed in the sandy loam soil amended with ANDB showed the same behaviour as in the clay loam soil which was opposite to the ratio obtained in the laboratory incubation experiment.

Comparison between two soil types amended with ANDB

The amount of total N mineralised from urea-treated the clay loam and sandy loam soil is shown in Figure 5.29.

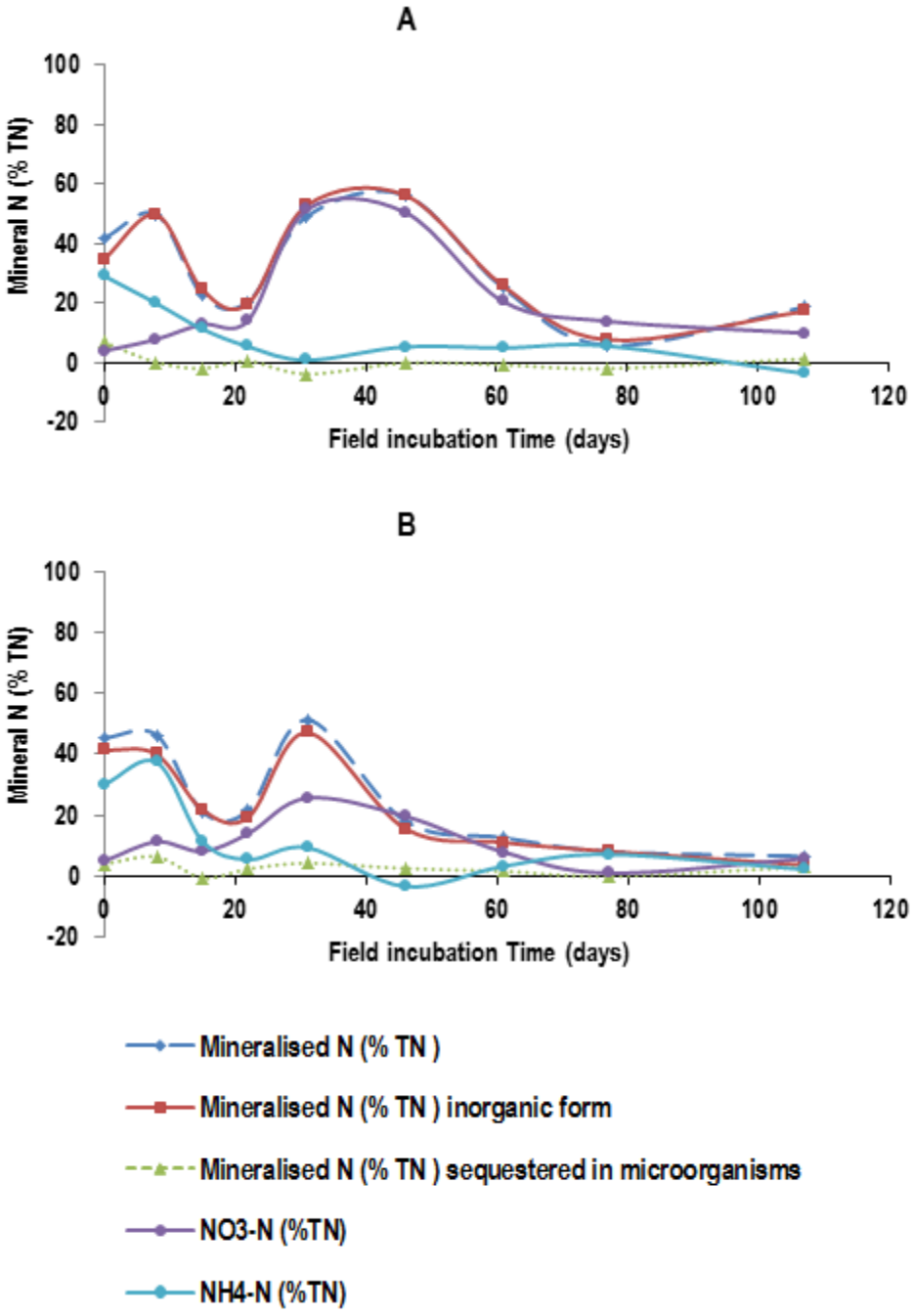

Figure 5.29. Mineral N (%TN added) from ANDB-applied to the clay loam (A) and sandy loam soil (B)

Mineralisation and nitrification process of ANDB-amended two soil types under different environmental condition are shown in Figure 5.8. As observed in the urea treatment, the nitrification processes in ANDB were greater in the laboratory incubation than in the field experiment (Table 5.11). It can be seen that, the amount

of organic-N mineralised in ANDB-amended the clay loam soil was greater than sandy loam soil.

Table 5.11 Mineral N from ANDB-amended two soil types under laboratory and field incubation trail

ANDB		Day of maximum mineralised N	% TN_{added} NH_4-N	%TN_{added} NO_3-N	%TN_{added} MBN	Mineralised N (% TN)	Organic-N mineralised (% org-N)
Clay loam soil	Lab	56	-1 ± 1	95 ± 3	10 ± 2	106 ± 19	105 ± 21
	Field	46	6 ± 1	51 ± 2	-0.03 ± 1	56 ± 2	54 ± 3
Sandy loam soil	Lab	56	1 ± 1	79 ± 3	3± 1	84 ± 9	78 ± 6
	Field	31	9 ± 1	26 ± 1	4.2 ± 1	51 ± 2	46 ± 2

The proportion of minerlisable N (% organic-N) was greater in the laboratory incubation than the field incubation experiment. By day 46, 53 % was mineralised from the organic-N contained in ANDB-amended the clay loam soil whereas 46 % was the maximum fraction released from the organic-N on day 31. The results from the current study are close to those obtained by Pu et al., (2008) in Queensland, where N mineralisation from anaerobic biosolids on a clay loam soil ranged between 44 and 59 %.

5.4.2.13 Mineral N dynamics of ADB in the clay loam soil

The rate of ammonification (NH_4-N) and nitrification (NO_3-N) processes of organic N in ADB-applied to the clay loam soil soils is shown in Figure 5.30.

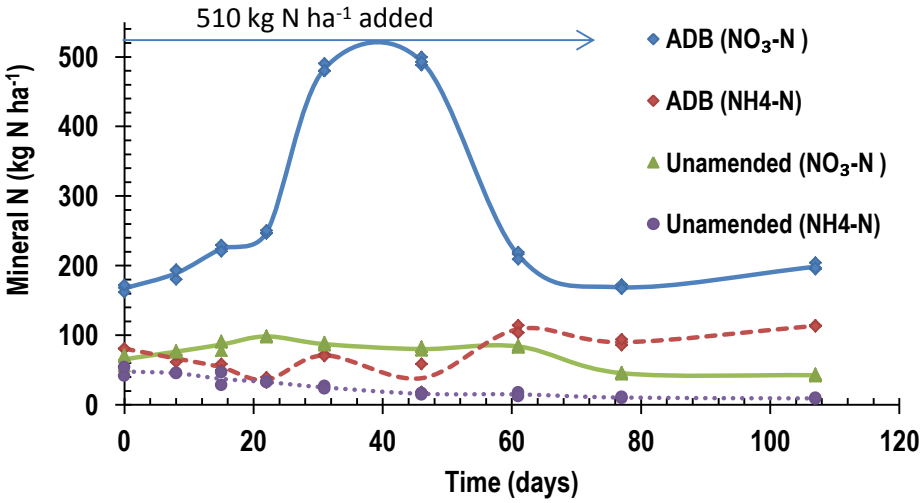

Figure 5.30. The effect of applying ADB on NO_3-N and NH_4-N dynamics on the clay loam soil (n=3) (the connecting lines show the mean of triplicate measurements plotted against times)

Production of mineral N (NH_4-N and NO_3-N) from ADB

When ADB was applied to the clay loam soil, the concentration of NH_4-N reached a maximum value of 114 kg N ha^{-1} on day 61. On this day, the difference of the quantity of NH_4-N observed in this biosolids was 97 kg N ha^{-1} which is approximately three times higher than the unamended clay loam soil.

At day 46, the level of NO_3-N recorded in ADB-amended clay loam soil was 493 kg N ha^{-1}. The NO_3-N released from ADB was greater than the unamended control plot. Also, the rate of nitrification was 11 kg N $ha^{-1}day^{-1}$. However, NO_3-N levels decreased between days 46 – 107. Possiblly due to loss of N through leaching process or the production of some organic-N compounds that were not measured.

5.4.2.14 Microbial biomass N and C

The concentrations of MBN and MBC in ADB-amended the clay loam soil over 107 days are presented in Figure 5.31.

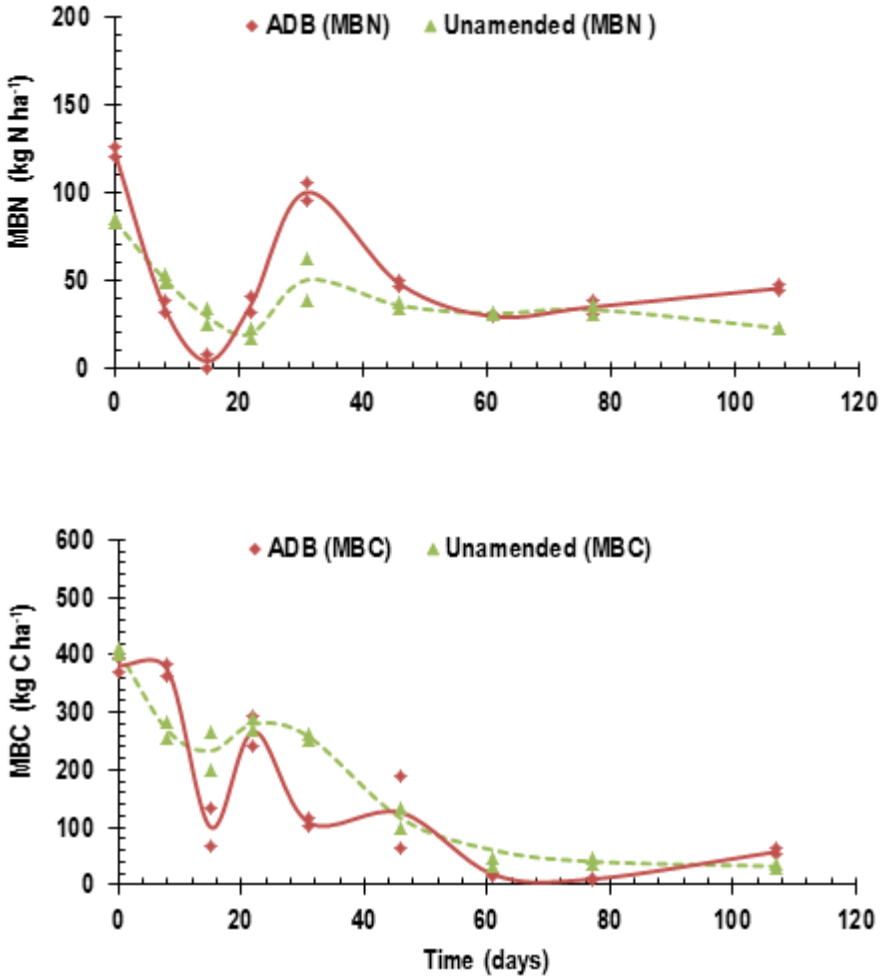

Figure 5.31. The influence of applying ADB on MBN and MBC dynamics on clay loam soil (n=2) (the connecting lines show the mean of duplicate measurements plotted against times)

The level of MBN in ADB was highest at 125 kg N ha^{-1} and then decreased to 4 kg N ha^{-1} on day 15. However, after day 15 onwards MBN the concentration increased to 100 kg N ha^{-1} on day 31 and started to decrease until the end of the experiment. The highest MBN concentration in unamended control soil was 80 kg N ha^{-1} by day 31.

The concentration of MBC was higher (391 kg C ha^{-1}) following the application of ADB and then decreased to 100 kg C ha^{-1} after two weeks. After that, the concentration of MBC increased to 267 kg C ha^{-1} on day 22. The increase of MBC might be as a result of increasing moisture content as shown in Figure 5.8. However, after day 22 forwards MBC showed a decreasing trend over the end of trial period. The level of MBC in the unamended control plot was above MBC levels found in ADB between days 15 – 22 and then started to decrease.

Microbial biomass ratio

Microbial biomass C to N ratio in ADB-amended the clay loam soil over 107 days is shown in Figure 5.32. The ratio of MBC: MBN was higher on day 15 and then decrease over 107 day. However, the MBC: MBN ratio observed in the unamended control soil was higher on day 22.

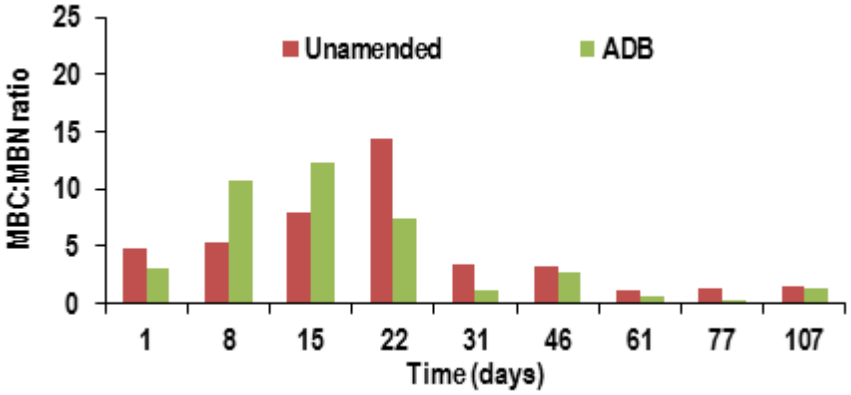

Figure 5.32. The ratio of microbial biomass C to N (mean, n = 3) in the ADB-treated clay loam soil

The same increasing than decreasing pattern was observed with ADB as with ANDB with the highest values recorded on day 22.

5.4.2.15 Mineral N dynamics of ADB in the sandy loam soil

The rates of ammonification (NH_4-N) and nitrification (NO_3-N) processes of organic N in ADB-applied to the sandy loam soil soils are shown in Figure 5.33.

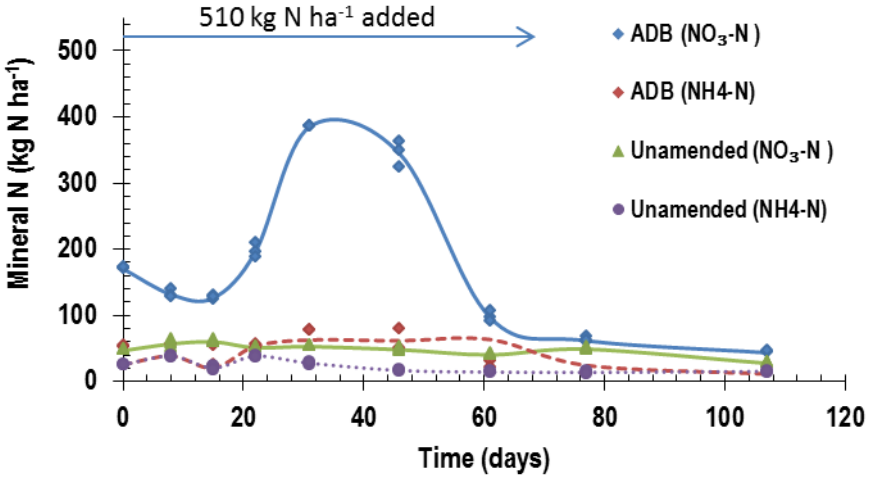

Figure 5.33. The effect of applying ADB on NO_3-N and NH_4-N dynamics on the sandy loam soil (n=3) (the connecting lines show the mean of triplicate measurements plotted against times)

Production of mineral N (NH_4-N and NO_3-N) from ADB

The concentrations of NH_4-N produced in ADB-amended sandy loam soil and unamended control soil were relatively consistent with unamended sandy loam soil over 107 days.

The greatest NO_3-N concentration observed in ADB was 384 kg N ha^{-1} on day 31. The difference observed between ADB and unamended control soil was 332 kg N ha^{-1} which is greater than the unamended control soil. Nitrification rate was 12 kg N ha^{-1} day^{-1} which close to ADB recorded in clay loam soil amendment.

There was a significantly ($P < 0.05$) higher in nitrification process in two biosolids-amended sandy loam soil compared with unamended control plot.

5.4.2.16 Microbial biomass N and C

The concentration of MBN and MBC in ADB-amended the sandy loam soil over 107 days is presented in Figure 5.34.

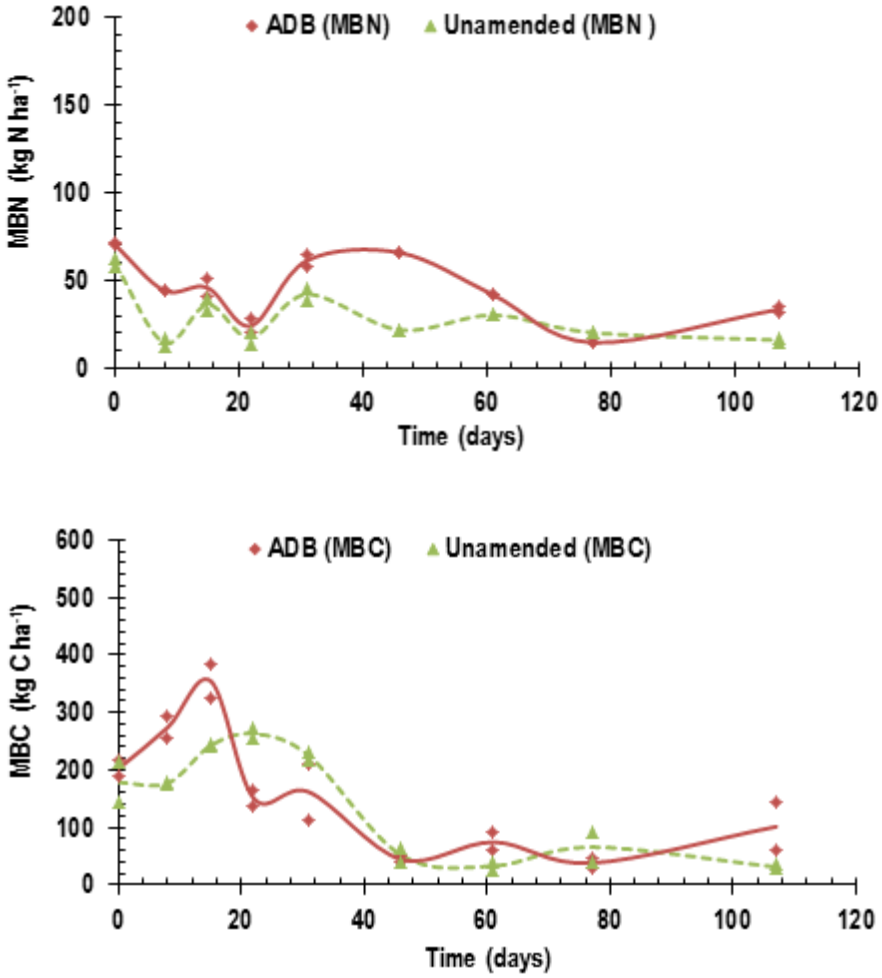

Figure 5.34. The influence of applying ADB on MBN and MBC dynamics on sandy loam soil (n=2) (the connecting lines show the mean of duplicate measurements plotted against times)

The greatest concentration of MBN was 69 kg N ha^{-1} in ADB by day 1 and then the MBN level decreased. The concentration of MBN in unamended sandy loam soil was below the MBN in ADB during the experiment period.

The levels of MBC reached a maximum values to 326 mg C kg^{-1} in ADB-amended sandy loam soil on day 15. At this point, the amount of MBC content in ADB was 36 % higher than the mass of MBC content observed in the unamended control soil. The levels of MBC in ADB indicated a decreasing trend over 107 days.

Microbial biomass ratio

Microbial biomass C to N ratio in ADB-amended sandy loam soil includes unamended control plots over 107 days are shown in Figure 5.35. The ratio of MBC:

MBN was higher on day 15 while the highest ratio of C: N microbial biomass observed in unamended control soil on day 22.

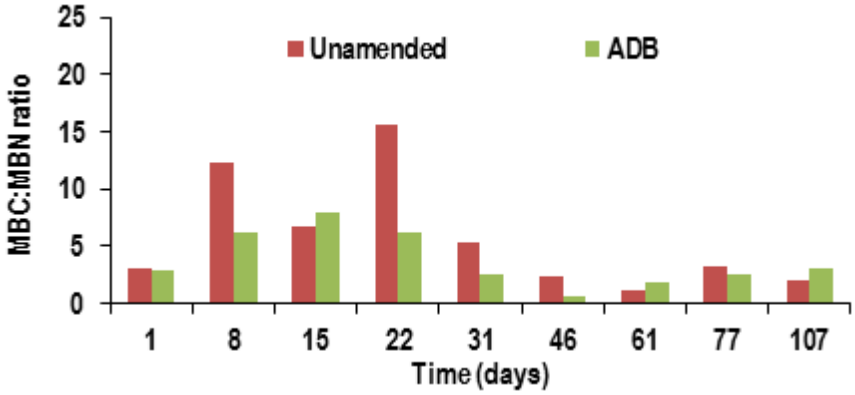

Figure 5.35. The ratio of microbial biomass C to N (mean, n = 3) in the ADB-treated sandy loam soil

The same behaviour of the MBC: MBN ratio observed in the clay loam soil. Therefore, a similar pattern was obtained under two different conditions.

Comparison between two soil types amended with ADB

The mineral N (%TN added) mineralised from the clay loam and sandy loam soil amended with ADB is shown in Figure 5.36.

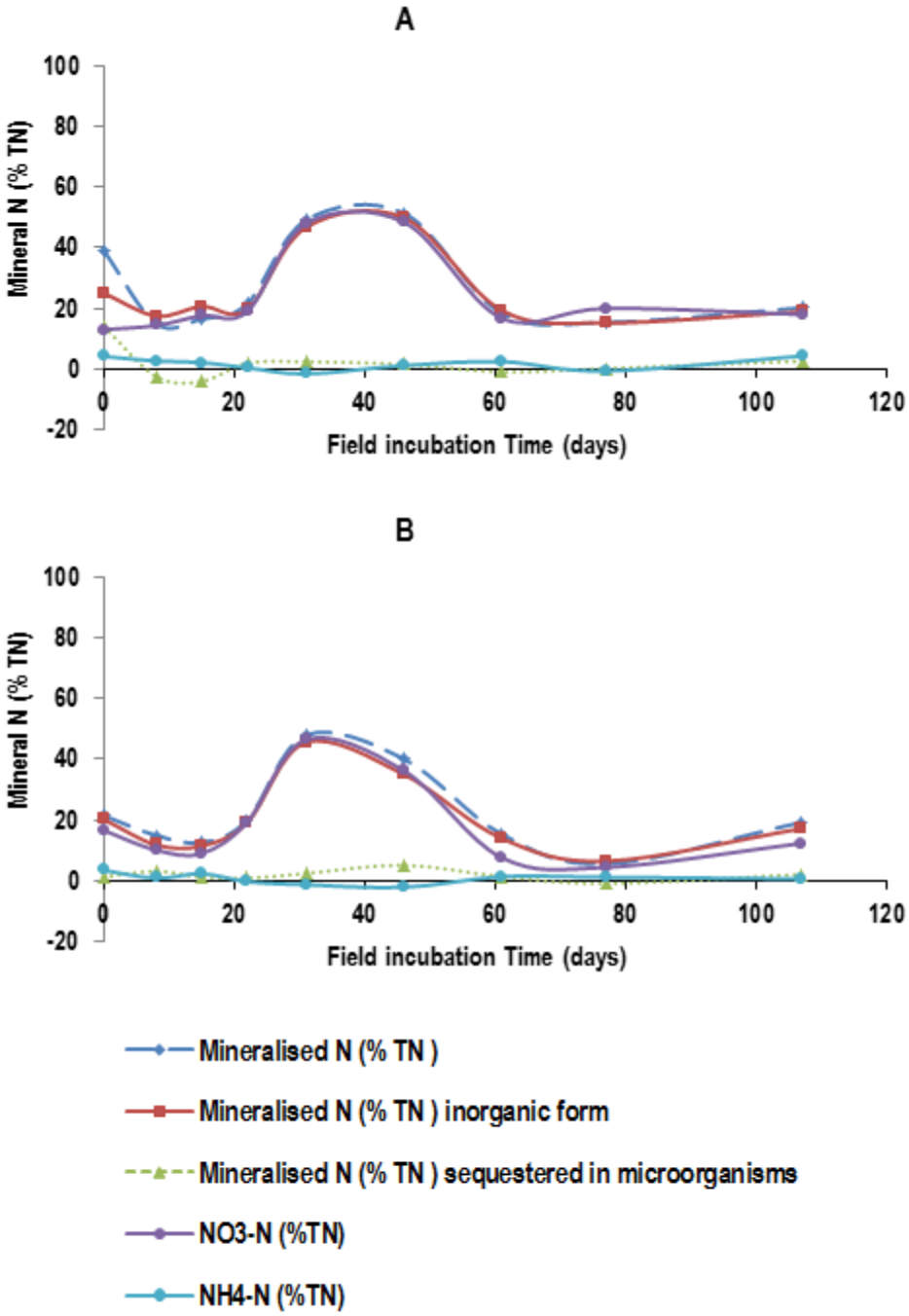

Figure 5.36. Mineral N (%TN) from ADB-applied to the clay loam (A) and sandy loam soil (B)

The maximum proportions of N mineralised from ADB-amended soils in the laboratory incubation and field incubation experiment are presented in Table 5.12. In clay loam soil types receiving ADB, the nitrification rate was approximately double under laboratory incubation than field incubation. However, the amount of N mineralised (%TN) in ADB-amended the clay loam soil under filed conditions was

double but it was greater in the sandy loam under field condition than field conditions.

Table 5.12 Mineral N from ADB-amended two soil types under laboratory and field incubation trail

ADB		Day of maximum mineralised N	% TN_{added} NH_4-N	%TN_{added} NO_3-N	%TN_{added} MBN	Mineralised N (% TN)	Organic-N mineralised (% org-N)
Clay loam soil	Lab	56	-2 ± 1	80 ± 3	16 ± 3	94 ± 4	102 ± 3
	Field	46	1 ± 0	49 ± 1	2 ± 1	51 ± 2	45 ± 1
Sandy loam soil	Lab	56	0.1 ± 1	41 ± 2	9 ± 2	49 ± 3	41 ± 3
	Field	31	-1± 1	47 ± 2	2 ± 0	48 ± 1	39 ± 1

(± Figure of means n=3)

The fraction of organic-N mineralised in ADB-amended the clay loam soil was greater than the amount observed in the sandy loam soil under both conditions. However, the proportion of the organic-N mineralised under field conditions was closed to the amount of organic-N contained in the aerobic biosolids amended soil reported by Rigby et al., (2010) in Western Australia.

5.4.3 Effect of amendments on mineral N

The ammonification and nitrification process in NH_4Cl and urea-amended soils were greater in the clay loam soil than in the sandy loam soil which is similar to the result obtained in the laboratory incubation experiment.

The ammonification process with ANDB was more than double that of ADB. This supported the theory that the stockpiling period had a significant effect on N availability from ADB.

On the other hand, by day 46, the application of both biosolids lead to a significant release of NO_3-N 474 kg N ha^{-1} from ANDB and 493 kg N ha^{-1} from ADB on the amended clay loam soil. The production of NO_3-N was significantly ($P < 0.001$) higher than the unamended control plots.

In sandy loam soil receiving ANDB and ADB, the greatest amount of NH_4-N released from ANDB was 292 kg N ha^{-1} on day 1 and 77 kg N ha^{-1} on day 46. The quantity of NH_4-N that was available from ANDB was about three times greater than ADB amended the same soil type.

Greatest amount of NO_3-N was available from the ANDB-amended sandy loam soil was 245 kg N ha^{-1} on day 31 but by day 46, after following the application of ADB into sandy loam soil, there was 384 kg N ha^{-1} amount released.

The two types of biosolids materials (anaerobically and aerobically digested biosolids) behaved quite differently in the soil. The decline in N availability over time was reasonably consistent within biosolids type, allowing the effects of cumulative applications to be predicted with quantity accuracy. Based on the results of this study, it is possible that this initial sequestration of NO_3-N could be due to increased microbial activity attributable to the differences in soil texture and soil fertility as suggested by Smith and Tibbett (2004) the lowest foliar N and soil NO_3-N concentrations in sludge, possibly immobilised as the large oxidisable C component of this sludge was metabolised by the microbial biomass.

5.4.4 Effect of soil type on mineral N

The addition of the commercial fertilisers (NH_4Cl and urea) to the clay loam soil produced the highest amount of NO_3-N on day 46. As a result of soil fertility as reported in the previous research (Keeney and Nelson, 1982, Ajwa and Tabatabai, 1994, Eriksen et al., 1999, Smith and Durham, 2002, Morris et al., 2003, Huang and Chen, 2009).

The amount of mineral N (NH_4-N and NO_3-N) released from the ANDB amended clay loam soil was greater than the sandy loam soil.

With ADB, the quantity of NH_4-N observed in both soil types was greatest in the clay loam soil. The amount of NO_3-N observed in ADB-amended the clay loam soil was 28 % greater than the sandy loam soil. This comparison between soil types showed that there was a significant effect of soil types on N mineralised from these materials. Others have seen similar patterns (Epstein et al., 1978, Parker and Sommers, 1983, Garau et al., 1986, Serna and Pomares, 1992).

5.4.5 Effect of biosolids and soil types on MBN

In the clay loam soil, the quantity of N immobilised from ANDB was 17 % greater than the MBN content in ADB on day 31. This is close to the values calculated in laboratory incubation experiment. The amount of MBN detected in ANDB-amended sandy loam soil was 10 % higher than the ADB-applied in the sandy loam soil which is consistent with laboratory incubation experiment. These results confirmed that biosolids types on microbial biomass N.

To investigate the effect of soil types on microbial biomass N, it is apparent that the quantities of MBN content in ANDB-amended two soil types was greater in the clay loam than sandy loam soil receiving similar application from both biosolids types. This was consistent with the observation recorded in the laboratory incubation experiment.

5.4.6 Effect of biosolids and soil types on microbial biomass C

It well known that the growth of soil microbial biomass has strong relationship to the amount of organic matter added to the soil (Jedidi et al., 2004, Fernández et al., 2007, Plaza et al., 2007, Cayuela et al., 2008, Rigby et al., 2009). In comparison between ANDB and ADB-amended clay loam, the amount observed was 13 % more in the ANDB-treated plots than in the ADB-treated plots. Similar results were observed in the sandy loam soil receiving the two biosolids (20 % greater in ANDB than ADB). There were differences in microorganism population possilly due to the background of the soils organic matter (Brookes and McGrath, 1984, Dar, 1996).

The MBC concentrations in clay loam soil amended with ANDB was 31 % greater than ADB whereas in the sandy loam soil, the amount of MBC found in ANDB was 38 % higher than ADB. There was no significant effect of soil types on microbial biomass C.

5.5 Conclusion

Understanding N release proportion of soils receiving biosolids is crucially important, as it helps to accurately quantify the mineralisable portion of the organic-N present in the biosolids. The current study showed that there was an effect of biosolids types in mineralisation N observed in different soil types. There was a greater nitrification rate

observed in ADB-amended in clay loam soil compared with ANDB without the influence of vegetation. On the other hand, ammonification rate was in greater in ANDB than ADB which may be due to losses of NH_4-N during the stockpiling period.

The results obtained in this field investigation as two biosolids to amend two soil types indicated that there were higher productions of NO_3-N in ADB than ANDB. This was indeed demonstrated by the amount of mineral N in ADB was greater compared to ANDB.

The clay loam soil produred greater amount of microbial biomass C and N in the clay loam.

The highest mineralised N measured in ANDB-amended in clay loam soil was 54 % compared to 45 % in ADB by day 46 which indicated that there was more N available in ANDB than ADB.

When the two biosolids were applied to the sandy loam soil, there was a greater amount of N mineralisable in ANDB (46 %) and ADB (39 %) after 31 days following the application of biosolids.

Under the same field conditions and similar amount of the application rates from two biosolids types amended the clay loam and sandy loam soil as described in section 5.2, with the presence of ryegrass were established to investigate the effect of ryegrass on N mineralisation rate as described in following chapter (6).

6

6 An Investigation into the Mineralisation Rates of Nitrogen and Immobilisation of Carbon and Nitrogen in Soil Amended with Biosolids under Field Conditions: vegetation

6.1 Introduction

This chapter analyses the plots shown in the experimental design chapter 5 (Section 5.2) on which perennial ryegrass was sown.

The aim of the field experiment was to investigate the effect of ryegrass on the mineral N process and to calculate the fraction of organic-N mineralised from two biosolids-amended soils. The effect of ryegrass on the microbial biomass C and N in the plots having received the two biosolids was examined. In this experiment, there were no plots with ryegrass which received either NH_4Cl or urea. This chapter only examines the effect of adding ANDB and ADB.

6.2 Mineralisation of N and N recoveries

The fractions of organic-N contained in ANDB and ADB applied to the clay loam and sandy loam soil were calculated using the same formula as described in the in chapter 4 (Section 4.2.5).

6.3 Results and Discussion

6.3.1 Mineral N dynamics under field conditions

Nitrogen is one of significant nutrients for plants' growth and development. Soil available N may limit plants growth in many ecosystems. N transformation in the soil is the most important and active process for N cycling in ecosystems. The soil N transformation is very complex because it is often affected by many factors, such as climatic conditions (mainly temperature and moisture), litter, chemical composition, soil pH and C/N, plant generated inhibitors, soil animals and nutrients (Barry, 2006, Pu et al., 2012, Wang et al., 2012).

The amount of N mineralised in the clay loam and sandy loam soils receiving two different biosolids and fertiliser during the field experiment are shown in Table 6.1and Table 6.2.

Table 6.1 Quantities of mineralisation rate of two biosolids and fertiliser types amended a clay loam soil with vegetation of ryegrass

Time (days)	Treatment types	NO_3-N (mg kg^{-1})	NH_4-N (mg kg^{-1})	MBN (mg kg^{-1})	Mineral N recoveries (mg kg^{-1})*	Mineralised N (% TN)	Mineralised N (%TN) inorganic form	Mineralised N (%TN) (sequestered in microorganism)	Mineralised N (% organic-N)
1	Unamended	32 ± 3	21 ± 4	37 ± 1					
	ANDB	43 ± 1	146 ± 4	65 ± 1	164	48.42	41.32	7.10	45.12
	ADB	73 ± 2	35 ± 0	111 ± 2	129	38.19	24.28	13.91	29.67
8	Unamended	33 ± 3	21 ± 2	22 ± 1					
	ANDB	70 ± 7	94 ± 0	22 ± 1	109	32.37	32.51	-0.14	27.92
	ADB	69 ± 5	24 ± 11	16 ± 1	33	9.83	12.21	-2.38	-2.81
15	Unamended	26 ± 1	19 ± 2	23 ± 3					
	ANDB	75 ± 1	127 ± 1	31 ± 4	166	48.97	46.64	2.33	45.65
	ADB	82 ± 1	24 ± 5	29 ± 3	67	19.86	18.21	1.65	8.63
22	Unamended	35 ± 2	24 ± 7	12 ± 3					
	ANDB	98 ± 1	130 ± 0	15 ± 3	172	50.87	49.94	0.93	47.69
	ADB	92 ± 1	28 ± 1	4 ± 1	52	15.44	18.22	-2.78	3.59
31	Unamended	18 ± 4	20 ± 6	47 ± 6					
	ANDB	130 ± 3	96 ± 3	34 ± 3	175	51.69	56.02	-4.33	48.61
	ADB	134 ± 7	33 ± 2	30 ± 10	112	33.09	38.88	-5.78	23.82
46	Unamended	22 ± 2	17 ± 1	18 ± 0					
	ANDB	129 ± 2	77 ± 1	29 ± 0	178	52.77	49.60	3.17	49.67
	ADB	120 ± 2	33 ± 2	48 ± 2	144	42.67	35.26	7.42	34.67

Cont. Table.

Time (days)	Treatment types	NO_3-N (mg kg^{-1})	NH_4-N (mg kg^{-1})	MBN (mg kg^{-1})	Mineral N recoveries (mg kg^{-1})*	Mineralised N (% TN)	Mineralised N (%TN) inorganic form	Mineralised N (%TN) (sequestered in microorganism)	Mineralised N (% organic-N)
	Unamended	13 ± 25	25 ± 7	18 ± 3					
61	ANDB	109 ± 3	64 ± 0	11 ± 1	127	37.53	39.82	-2.30	33.37
	ADB	83 ± 2	58 ± 2	14 ± 0	98	29.07	30.38	-1.31	19.12
	Unamended	10 ± 2	31 ± 3	3 ± 0					
77	ANDB	25 ± 1	54 ± 1	42 ± 0	78	23.09	15.34	7.75	17.89
	ADB	26 ± 0	30 ± 10	12 ± 0	24	7.11	5.22	1.89	-6.04
	Unamended	15 ± 0	34 ± 6	7 ± 1					
107	ANDB	23 ± 1	44 ± 8	15 ± 1	26	7.56	5.82	1.74	1.33
	ADB	6 ± 0	49 ± 1	10 ± 0	9	2.61	1.99	0.62	-11.14

*Mineral N recoveries (NO_3-N, NH_4-N and MBN) after subtracted from the unamended control soil

Table 6.2 Quantities of mineralisation rate of two biosolids and fertiliser types amended a sandy loam soil with vegetation of ryegrass

Time (days)	Treatment types	NO_3-N (mg kg^{-1})	NH_4-N (mg kg^{-1})	MBN (mg kg^{-1})	Mineral N recoveries (mg kg^{-1})*	Mineralised N (% TN)	Mineralised N (%TN) inorganic form	Mineralised N (%TN) (sequestered in microorganism)	Mineralised N (% organic-N)
1	Unamended	27 ± 1	12 ± 0	27 ± 1					
	ANDB	37 ± 11	134 ± 3	42 ± 1	117	34.69	30.82	3.86	29.15
	ADB	77 ± 1	23 ± 1	32 ± 2	66	19.54	18.33	1.21	7.52
8	Unamended	27 ± 2	21 ± 1	8 ± 1					
	ANDB	62 ± 2	121 ± 8	31 ± 1	129	38.24	32.31	5.93	32.79
	ADB	66 ± 4	17 ± 5	18 ± 0	45	13.37	10.86	2.50	0.65
15	Unamended	19 ± 3	5 ± 1	6 ± 2					
	ANDB	56 ± 1	130 ± 7	46 ± 5	161	47.72	38.38	9.34	42.52
	ADB	71 ± 2	22 ± 0	18 ± 3	80	23.66	20.62	3.04	11.95
22	Unamended	21 ± 1	22 ± 2	6 ± 1					
	ANDB	63 ± 1	103 ± 0	17 ± 3	130	38.59	35.64	2.95	33.13
	ADB	83 ± 3	26 ± 6	8 ± 2	67	19.94	19.42	0.52	7.90
31	Unamended	17 ± 2	24 ± 2	20 ± 2					
	ANDB	96 ± 0	89 ± 9	27 ± 3	150	44.28	42.84	1.45	39.06
	ADB	105 ± 3	27 ± 1	27 ± 2	99	29.19	27.22	1.97	18.18
46	Unamended	8 ± 3	28 ± 4	9 ± 0					
	ANDB	60 ± 5	98 ± 8	12 ± 1	125	36.92	35.96	0.95	31.39
	ADB	102 ± 11	28 ± 0	25 ± 1	111	32.99	28.76	4.23	22.32

Cont. Table.

Time (days)	Treatment types	NO_3-N (mg kg^{-1})	NH_4-N (mg kg^{-1})	MBN (mg kg^{-1})	Mineral N recoveries (mg kg^{-1})*	Mineralised N (% TN)	Mineralised N (%TN) inorganic form	Mineralised N (%TN) (sequestered in microorganism)	Mineralised N (% organic-N)
61	Unamended	10 ± 2	33 ± 11	15 ± 5					
	ANDB	34 ± 1	103 ± 11	13 ± 1	91	27.01	27.59	-0.58	21.18
	ADB	38 ± 2	55± 10	6 ± 1	41	12.08	15.34	-3.26	-0.75
77	Unamended	3 ± 0	20 ± 5	6 ± 1					
	ANDB	8 ± 0	60 ± 8	7 ± 1	45	13.22	13.09	0.12	6.83
	ADB	4 ± 0	28 ± 2	6 ± 4	8	2.47	2.55	-0.08	-11.49
107	Unamended	2 ± 0	42 ± 5	5 ± 1					
	ANDB	45 ± 2	70 ± 6	21 ± 1	86	25.46	21.71	3.75	10.44
	ADB	31 ± 1	39 ± 4	14 ± 7	35	10.45	8.39	2.06	-6.71

*Mineral N recoveries (NO_3-N, NH_4-N and MBN) after subtracted from the unamended control soil

6.3.1.1 Mineral N dynamics of ANDB in the clay loam soil

The rate of ammonification (NH_4-N) and nitrification (NO_3-N) processes of organic-N in ANDB-applied to the clay loam soil soils is shown in Figure 6.1.

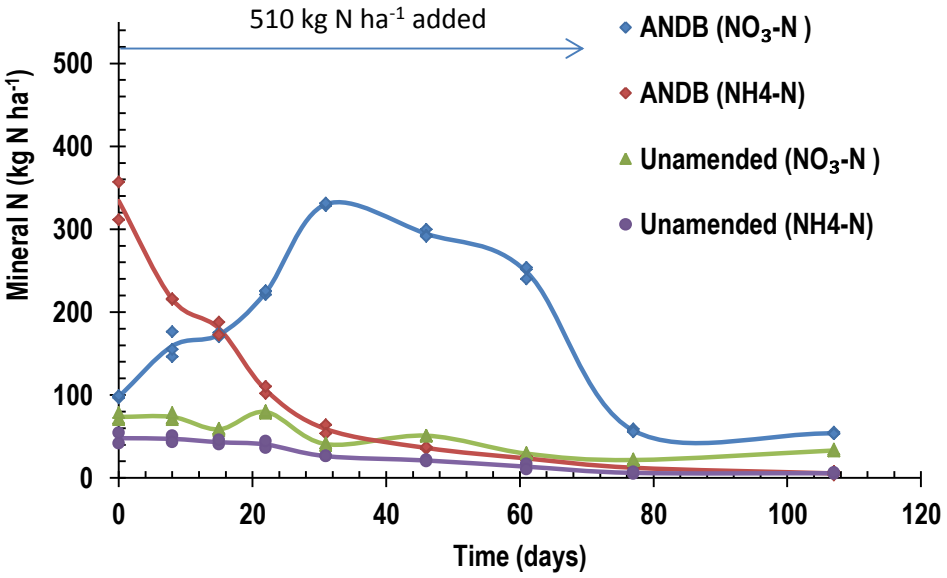

Figure 6.1. The effect of applying ANDB on NO_3-N and NH_4-N dynamics on the clay loam soil (n=3) (the connecting lines show the mean of triplicate measurements plotted against times)

Production of mineral N (NH_4-N and NO_3-N) from ANDB

The net NH_4-N produced in ANDB-amended soil between days 1 – 8, initially decreased from 311 kg N ha^{-1} to 176 kg N ha^{-1}; after that the level of NH_4-N decreased. The concentration of NH_4-N in unamended control plots was relatively constant throughout the period of the experiment.

In the clay loam soil amended with ANDB, the concentration of NO_3-N increased to 328 kg N ha^{-1} on day 31. Therefore, the quantity of NO_3-N produced between days 1 – 31 was greater than the NO_3-N values recorded in the unamended control plots. The process of nitrification during the period between days 1 – 31 was 3.93 kg N $ha^{-1}day^{-1}$ as the result of the favourable soil temperature (9.7 – 10 C) and soil moisture (23 – 27 %) conditions. Such soil conditions might have encouraged nitrification and increased mineralisation of organic-N in to inorganic-N fractions. However, from day 31 onwards levels of NO_3-N decreased. During the trial period (107 days), the level

of NO_3-N in unamended control plots was relatively constant. The reduction of NO_3-N may be due to loss of N through various ways such as denitrification or immobilisation or leaching or crop uptake.

6.3.1.2 Microbial biomass N and C

The concentration of MBN and MBC in ANDB-amended the clay loam soil over 107 days is presented in Figure 6.2

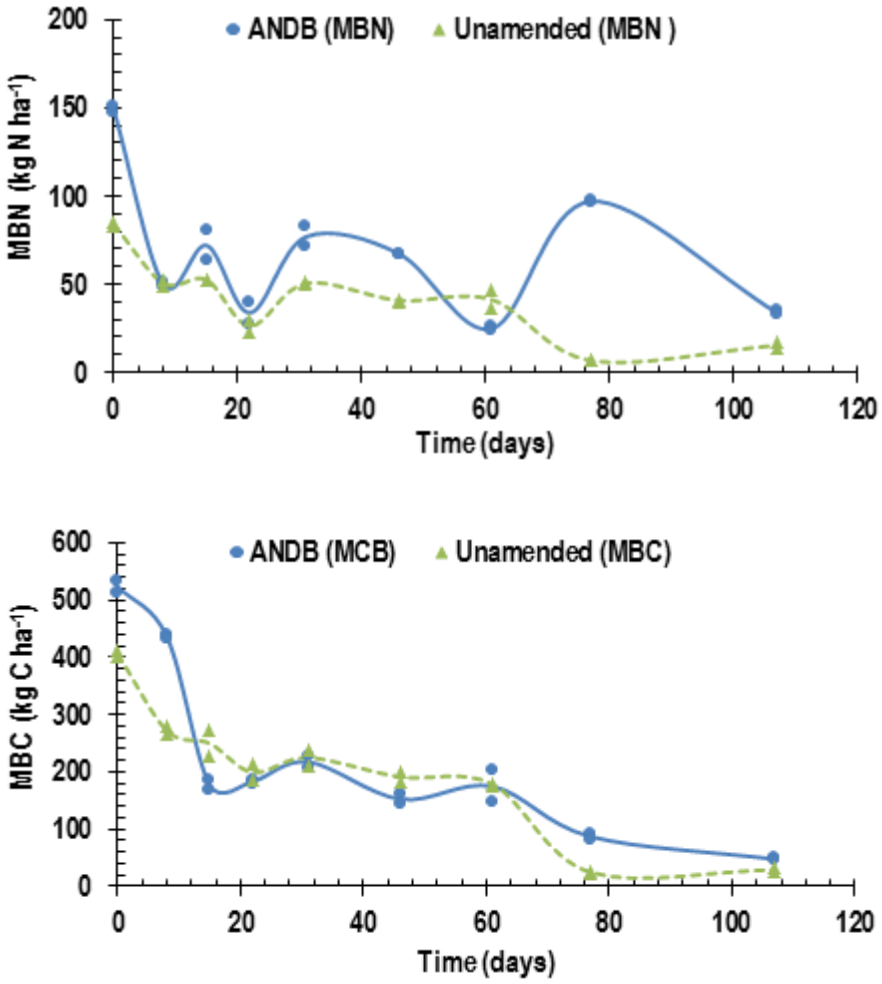

Figure 6.2. The influence of applying ANDB on MBN and MBC dynamics on clay loam soil (n=2) (the connecting lines show the mean of duplicate measurements plotted against times)

The highest MBN concentration in ANDB-amended in a clay loam soil with ryegrass was 149 kg N ha^{-1} following the application of ANDB. The concentration of MBN fluctuated between days 8 – 61 where the lower concentration of MBN (24 kg N ha^{-1})

observed after that increased to 97 kg N ha^{-1} on day 77and then decreased to 33 kg N ha^{-1} by day 107. The levels of MBN in unamended control plots were lower than in the ANDB-amended plot during the experiment period.

Microbial biomass carbon (MBC) concentration in ANDB-amended in a clay loam soil with ryegrass was initially higher (523 kg C ha^{-1}) than the MBC concentration measured in the unamended control plot and then decreased to 176 kg C ha^{-1} after two weeks following the application of biosolids; it fluctuated between days 15 – 107. Generally, MBC ranged between 300 – 1200 kg C ha^{-1} which is within the normal range as reported in the previous studies (Franco-Hernández et al., 2003, Jedidi et al., 2004, Fernandes et al., 2005). As observed previously, the clay loam soil was more fertile, with high organic matter and larger microbial biomass (See chapter 4, Table 4.6). Addition of the organic matter to soil will stimulate the soil microbes to decompose the organic N to inorganic forms which then becomes available for crops. This is consistent with results reported by Calbrix et al., (2007).

Microbial biomass ratio

Microbial biomass C to N ratio in ANDB-amended the clay loam soil over 107 days is shown in Figure 6.3. The highest ratio of MBC: MBN was observed on day 22 while on day 8, it was slightly higher than the ratio of microbial biomass C: N in the unamended control plot.

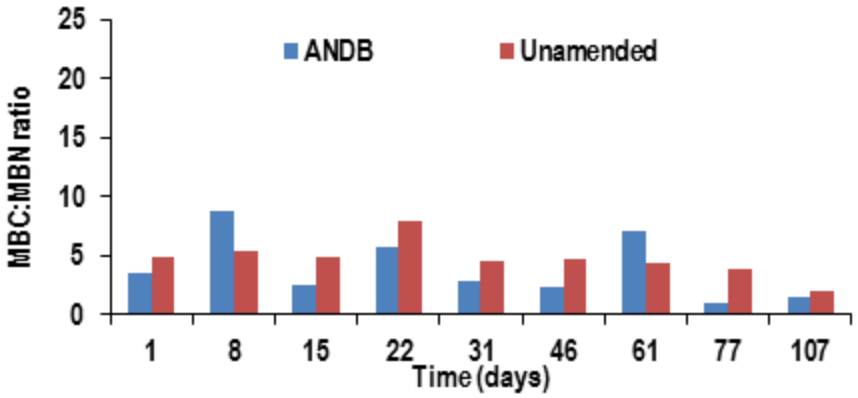

Figure 6.3. The ratio of microbial biomass C to N (mean, n = 3) in the ANDB treated clay loam soil

6.3.1.3 Mineral N dynamics of ANDB in the sandy loam soil

The rate of ammonification (NH_4-N) and nitrification (NO_3-N) processes of organic-N in ANDB-applied to the sandy loam soil soils is shown in Figure 6.4.

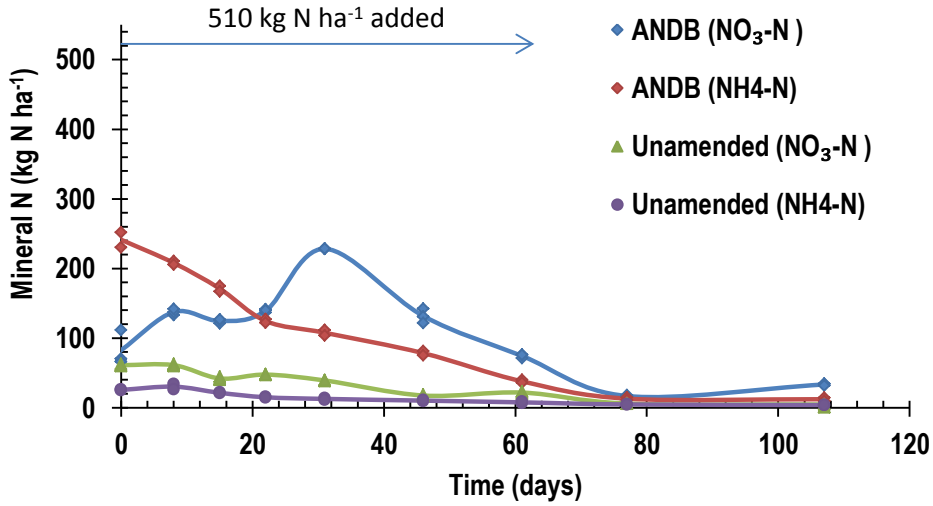

Figure 6.4. The effect of applying ANDB on NO_3-N and NH_4-N dynamics on the sandy loam soil (n=3) (the connecting lines show the mean of triplicate measurements plotted against times)

Production of mineral N (NH_4-N and NO_3-N) from ANDB

The level of NH_4-N observed in sandy loam soil amended with ANDB between days 1 – 107 decreased. The mass of NH_4-N in ANDB was higher than the unamended control plots. From day 15 onwards, the concentration of NH_4-N decreased. The concentration of NH_4-N in the unamended control plots was relatively constant during over the whole period of the experiment.

In the sandy loam soil amended with ANDB, the concentration of NO_3-N showed an increasing trend and reached a maximum (228 kg N ha^{-1}) by 31. The amount of NO_3-N recorded in this material was greater than the unamended control plots. The nitrification rate was 7 kg N ha^{-1} day^{-1}. After this period the level of NO_3-N decreased. Concentration of NO_3-N in the unamended control plot was relatively constant over 107 days which is similar to the behaviour in the clay loam soil.

6.3.1.4 Microbial biomass N and C

The concentration of MBN and MBC in ANDB-amended the sandy loam soil over 107 days is presented in Figure 6.5

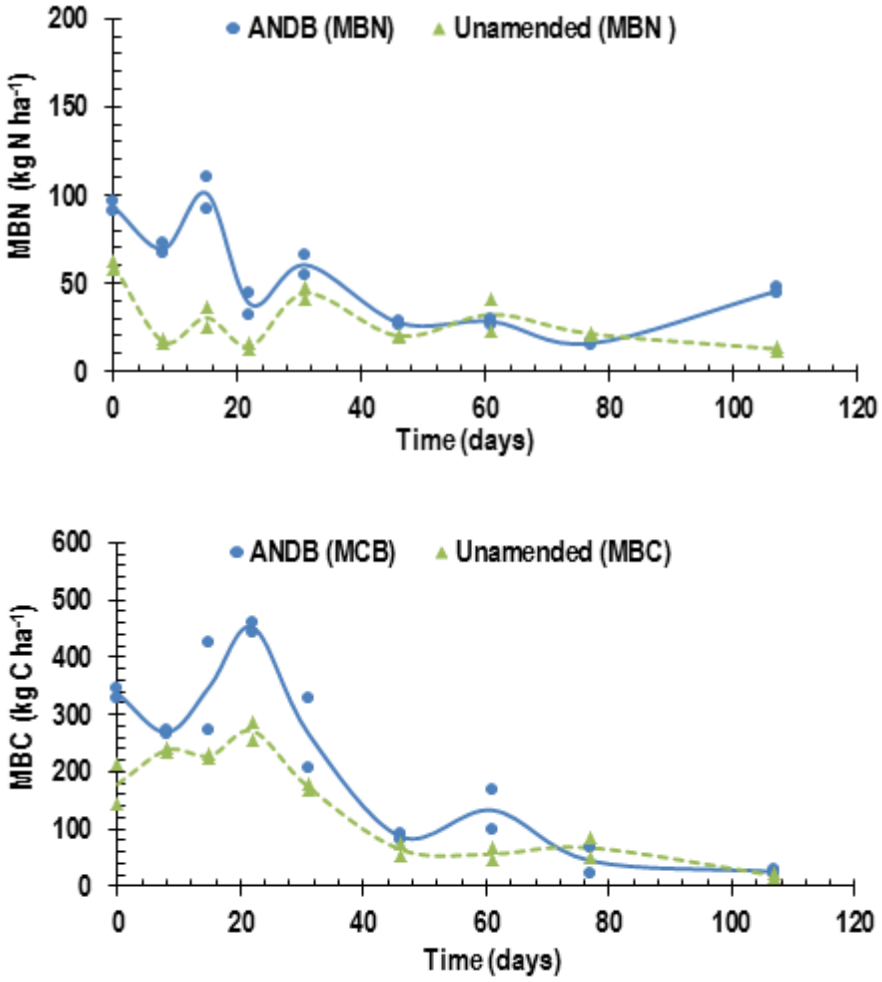

Figure 6.5. The influence of applying ANDB on MBN and MBC dynamics on sandy loam soil (n=2) (the connecting lines show the mean of duplicate measurements plotted against times)

The MBN concentration in the ANDB-amended sandy loam soil reached a maximum value of 101 kg N ha^{-1} by day 15 and then decreased.

In the sandy loam soil amended with ANDB of there was an increase in the concentration of MBC which reached a maximum value of 452 kg C ha^{-1} on day 22. After that it decreased quickly.

Microbial biomass ratio

Microbial biomass C to N ratio in ANDB amended the sandy loam soil over 107 days is shown in Figure 6.6. The ratio of MBC: MBN was highest on day 22 which is similar to the clay loam soil.

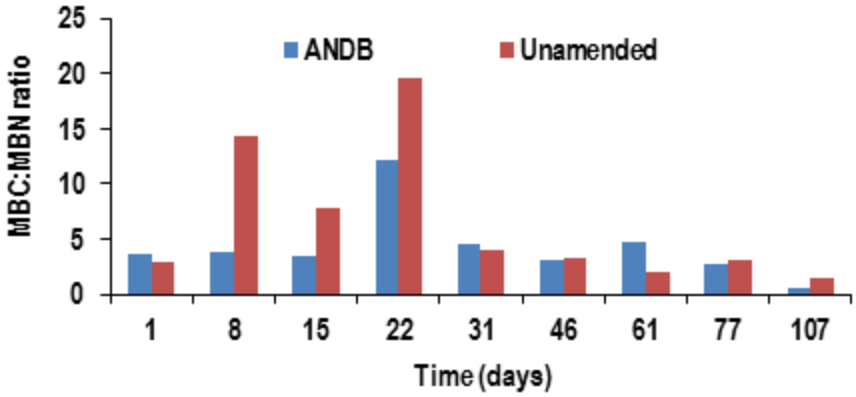

Figure 6.6. The ratio of microbial biomass C to N (mean, n = 3) in the ANDB treated sandy loam soil

Comparison between two soil types amended with ANDB

The total N mineralised (TN added), the total inorganic-N form and the amount of N immobilised by soil microbes are shown in Figure 6.7. The nitrification rate without ryegrass was greater in the clay loam soil. In sandy loam soil, the nitrification was higher with vegetation in early stage (15 days) compared to the treatment without vegetation. Overall, the amount of inorganic-N form was close to the total N mineralised (%TN added).

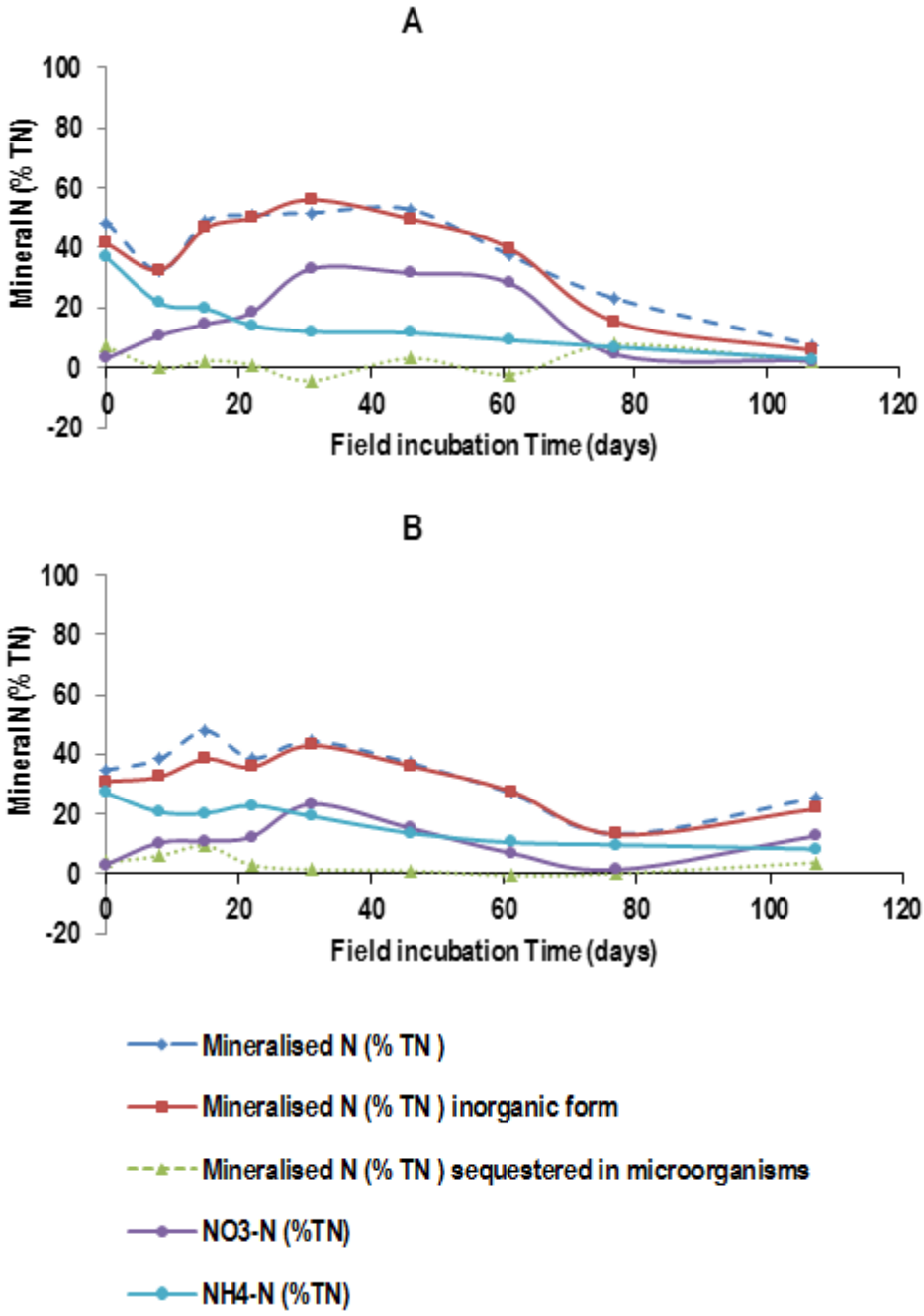

Figure 6.7. Mineral N (%TN added) from ANDB-applied to the clay loam (A) and sandy loam soil (B)

The maximum N mineralised from ANDB-amended the clay loam and sandy loam soil under field conditions without and with vegetation of the ryegrass are shown in Table 6.3. The amount of mineral N (% TN added) shows that there was no difference between without and with vegetation of the ryegrass, however, it can be seen that the quantities of mineral N with ryegrass is slightly lower than the amount

of total N mineral without vegetation of ryegrass presumably due to the ryegrass uptake of the available N. This is confirmed by Rigby (2008) where the amount of N mineralisation in the case of vegetation of ryegrass was less than without vegetation of ryegrass.

Table 6.3 Mineral N from ANDB-amended two soil types under field incubation trail

ANDB		Day of maximum mineralised N	$\%TN_{added}$ NH_4-N	$\%TN_{added}$ NO_3-N	$\%TN_{added}$ MBN	Mineralised N (% TN)	Organic-N mineralised (% org-N)
Clay loam soil	Field (no vegetation)	46	6 ± 1	51 ± 2	-0.03 ± 1	56 ± 2	54 ± 3
	Field (vegetation)	46	12 ± 2	32 ± 2	3 ± 1	53 ± 1.2	50 ± 1
Sandy loam soil	Field (no vegetation)	31	9 ± 1	26 ± 1	4 ± 1	51 ± 2	46 ± 2
	Field (vegetation)	15	25 ± 2	11 ± 1	9 ± 1	48 ± 1	43 ± 1

The proportion of organic-N mineralised without vegetation of ryegrass was greater than with vegetation of ryegrass in clay loam soil amended with ANDB.

The mineralisation of N observed in sandy loam soil amended with ANDB was closed in both case of ryegrass.

6.3.1.5 Mineral N dynamics of ADB in the clay loam soil

The changes of the NH_4-N and NO_3-N rates of organic N in ADB-applied to the clay loam soil are shown in Figure 6.8.

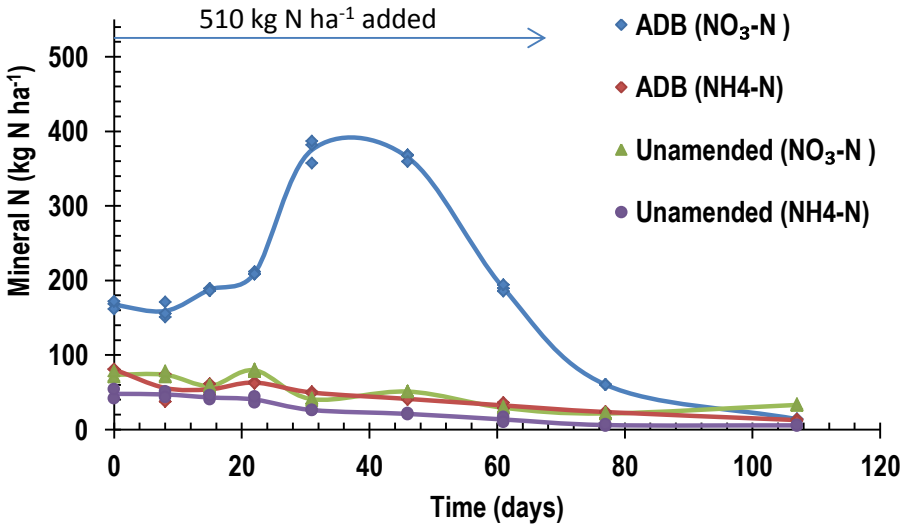

Figure 6.8. The effect of applying ADB on NO_3-N and NH_4-N dynamics on the clay loam soil (n=3) (the connecting lines show the mean of triplicate measurements plotted against times)

Production of mineral N (NH_4-N and NO_3-N) from ADB

The net NH_4-N produced in ADB-amended clay loam soil and unamended control plot was relatively constant over 107 days. Thus NH_4-N concentrations in two biosolids amended clay loam soil were substantially higher ($P < 0.001$) than the unamended control plots.

In the clay loam soil receiving ADB, the NO_3-N levels indicated an increasing trend and reached a maximum value of 375 kg N ha^{-1} on day 31. The production of NO_3-N observed in ADB between days 1 – 31 was higher than the unamended control plots. The nitrification process was 4.90 kg N ha^{-1} day^{-1}. However, the levels of NO_3-N demonstrated a decreasing trend between days 31 – 107. The statistical test using ANOVA revealed that the NO_3-N concentrations in two biosolids amended clay loam soil were significantly higher ($P < 0.001$) than the corresponding NO_3-N levels observed in the unamended control plots.

6.3.1.6 Microbial biomass N and C

The concentration of MBN and MBC in ADB-amended the clay loam soil over 107 days is presented in Figure 6.9.

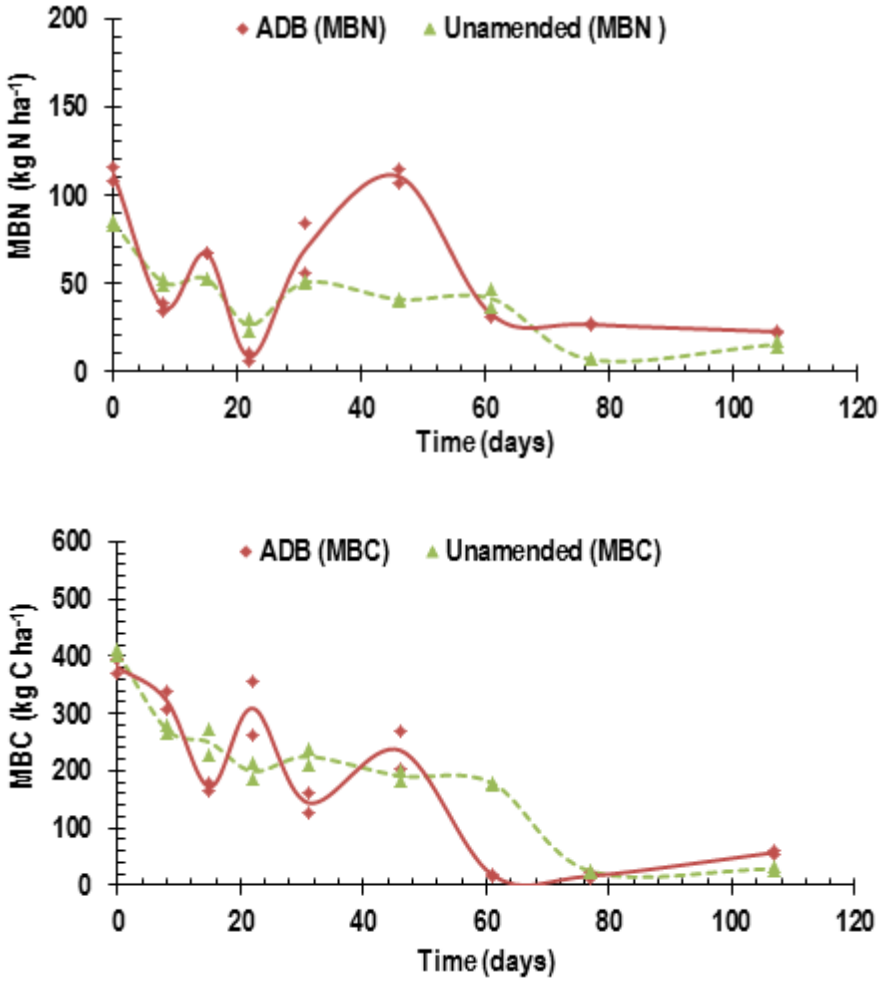

Figure 6.9. The influence of applying ADB on MBN and MBC dynamics on clay loam soil (n=2) (the connecting lines show the mean of duplicate measurements plotted against times)

In the clay loam soil receiving ADB the concentration of MBN was 112 kg N ha^{-1} on day 1 and decrease to 8 kg N ha^{-1} by day 22 followed by an increase to 111 kg N ha^{-1} on day 46. After this period MBN concentration indicated a decreasing trend over 107 day. The levels of MBN in unamended control plots were lower than ADB over 107 days.

The concentration of MBC also in ADB was greater (381 kg C ha^{-1}) at the beginning the experiment and then continued decrease to 171 kg C ha^{-1} which is similar with ANDB at the same day. Soil temperature recorded between days 1 – 15 increased whereas the moisture content decreased from 24.7 to 21.6 %. These results shows that the moisture content directly affects microbial activity and also on the other way moisture content affects indirectly the mineralisation of N and aerobic microbial

activity over monitoring the diffusion of oxygen in soil (Barbarika et al., 1985, Chen et al., 2009, Wang et al., 2012). Therefore, addition of organic matter contained in the biosolids lead to an increase in MBC the stimulation of soil microbial growth and breakdown the organic matter (Marinari et al., 2010).

Microbial biomass ratio

Microbial biomass C to N ratio in ADB-amended the clay loam soil over 107 days is shown in Figure 6.10. Greatest ratio of MBC: MBN observed from ADB and unamended control plots on day 22 which may be due to growth of fungi.

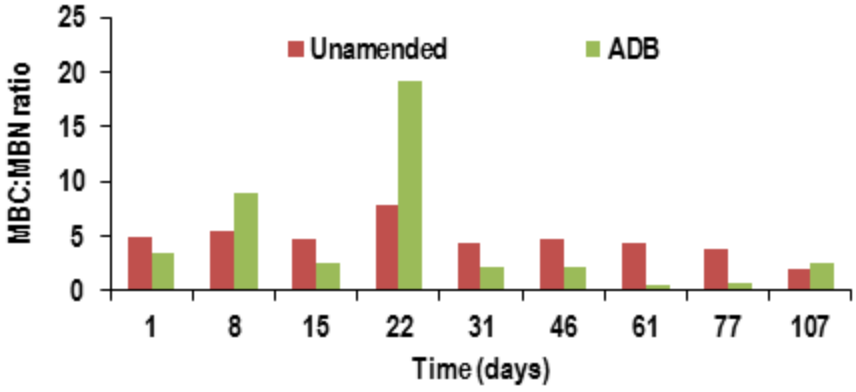

Figure 6.10. The ratio of microbial biomass C to N (mean, n = 3) in the ADB-treated clay loam soil

6.3.1.7 Mineral N dynamics of ADB in the sandy loam soil

The rate of ammonification (NH_4-N) and nitrification (NO_3-N) processes of organic-N in ADB-applied to the sandy loam soil soils is shown in Figure 6.11.

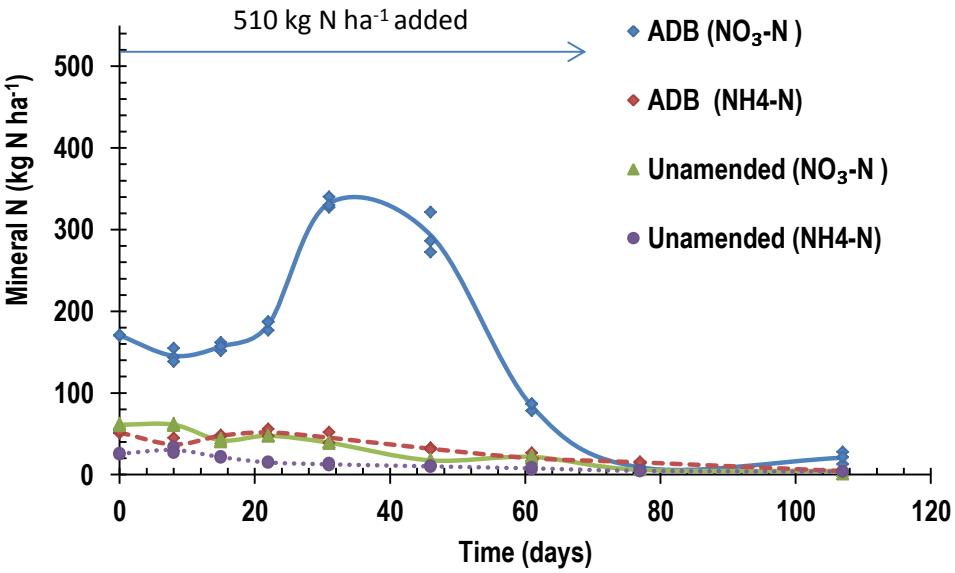

Figure 6.11. The effect of applying ADB on NO_3-N and NH_4-N dynamics on the sandy loam soil (n=3) (the connecting lines show the mean of triplicate measurements plotted against times)

Production of mineral N (NH_4-N and NO_3-N) from ADB

The levels of NH_4-N in ADB-amended sandy loam soil and unamended control plot were relatively constant over 107 days which is consistent with the clay loam soil. The level of NH_4-N values observed in ANDB-amended sandy loam soil was significantly ($P < 0.001$) higher than the levels in unamended control plots (Figure 6.4). There was no significant ($P > 0.05$) difference in the levels of NH_4-N between ADB and unamended control plots.

Likewise, NO_3-N concentrations in ADB-amended sandy loam soil indicated an increase to a maximum of 332 kg N ha^{-1} by day 31. The amount of NO_3-N obtained in biosolids on day 31 was higher than the amount observed in the unamended control plots. The rate of nitrification was 3.96 kg N ha^{-1} day^{-1}. However, after this point the levels of NO_3-N indicated a decreasing trend over 107 days. There was a significant difference ($P < 0.001$) in the concentration of NO_3-N in two biosolids-amended sandy loam soil and the concentration obtained in the unamended control plots.

6.3.1.8 Microbial biomass N and C

The concentration of MBN and MBC in ADB-amended the sandy loam soil over 107 days is presented in Figure 6.12.

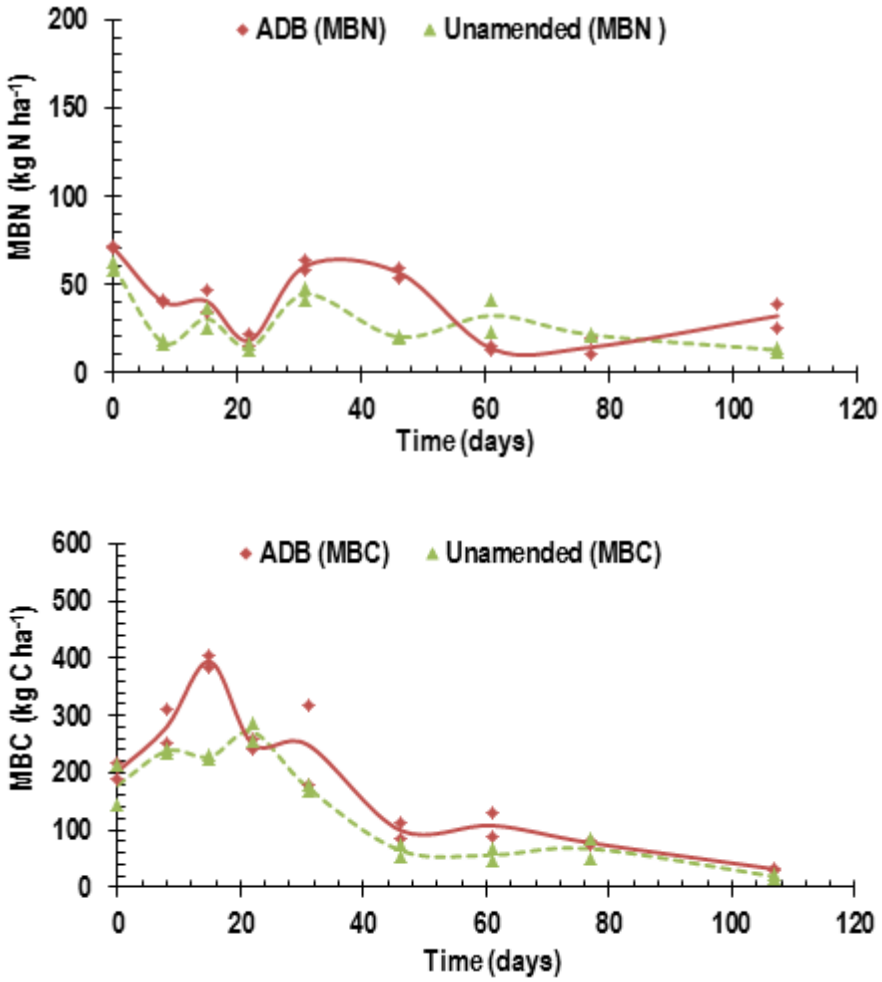

Figure 6.12. The influence of applying ADB on MBN and MBC dynamics on sandy loam soil (n=2) (the connecting lines show the mean of duplicate measurements plotted against times)

On the same sandy loam soil conditions treated with ADB, the level of MBN was lower than ANDB between days 1 – 22. However, the concentration of MBN was similar with ANDB on day 31 (60 kg N ha^{-1}) and then decrased over time. The levels of MBN observed in unamended control soil were lower than both biosolids during the trial period.

Similarly, the MBC concentration increased in the ADB-amended sandy loam soil reaching 394 kg C ha^{-1} between days 1 – 15 and then decreased. The level of MBC in unamended control plots with ryegrass decreased and remained below to the MBC in two biosolids-amended soils during the experiment period.

Microbial biomass ratio

Microbial biomass C to N ratio in ADB-amended the sandy loam soil over 107 days is shown in Figure 6.13. The ratio of MBC: MBN was highest in ADB and unamended control soils by day 22 which was also observed in the clay loam soil receiving the same biosolids.

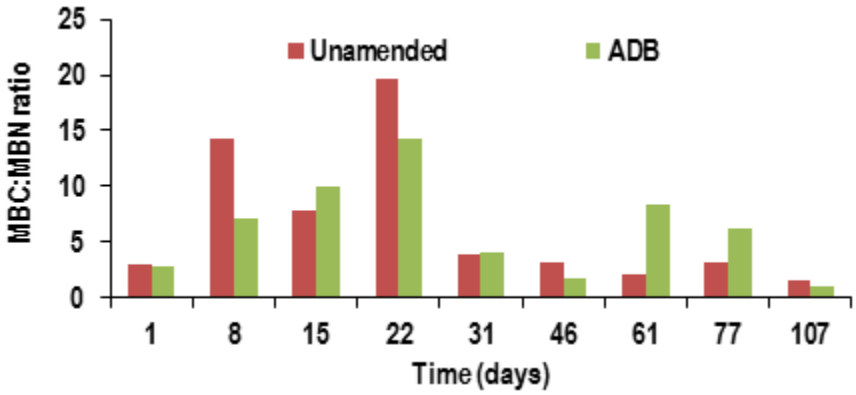

Figure 6.13. The ratio of microbial biomass C to N (mean, n = 3) in the ADB-treated sandy loam soil

Comparison between two soil types amended with ADB

The total N mineralised (TN added), the total inorganic-N form and the amount of N immobilised by soil microbes are shown in Figure 6.14. The nitrification rate without vegetation of ryegrass was greater in the clay loam soil. In sandy loam soil, the nitrification was higher with vegetation in early stage (15 days) compared to the treatment without vegetation.

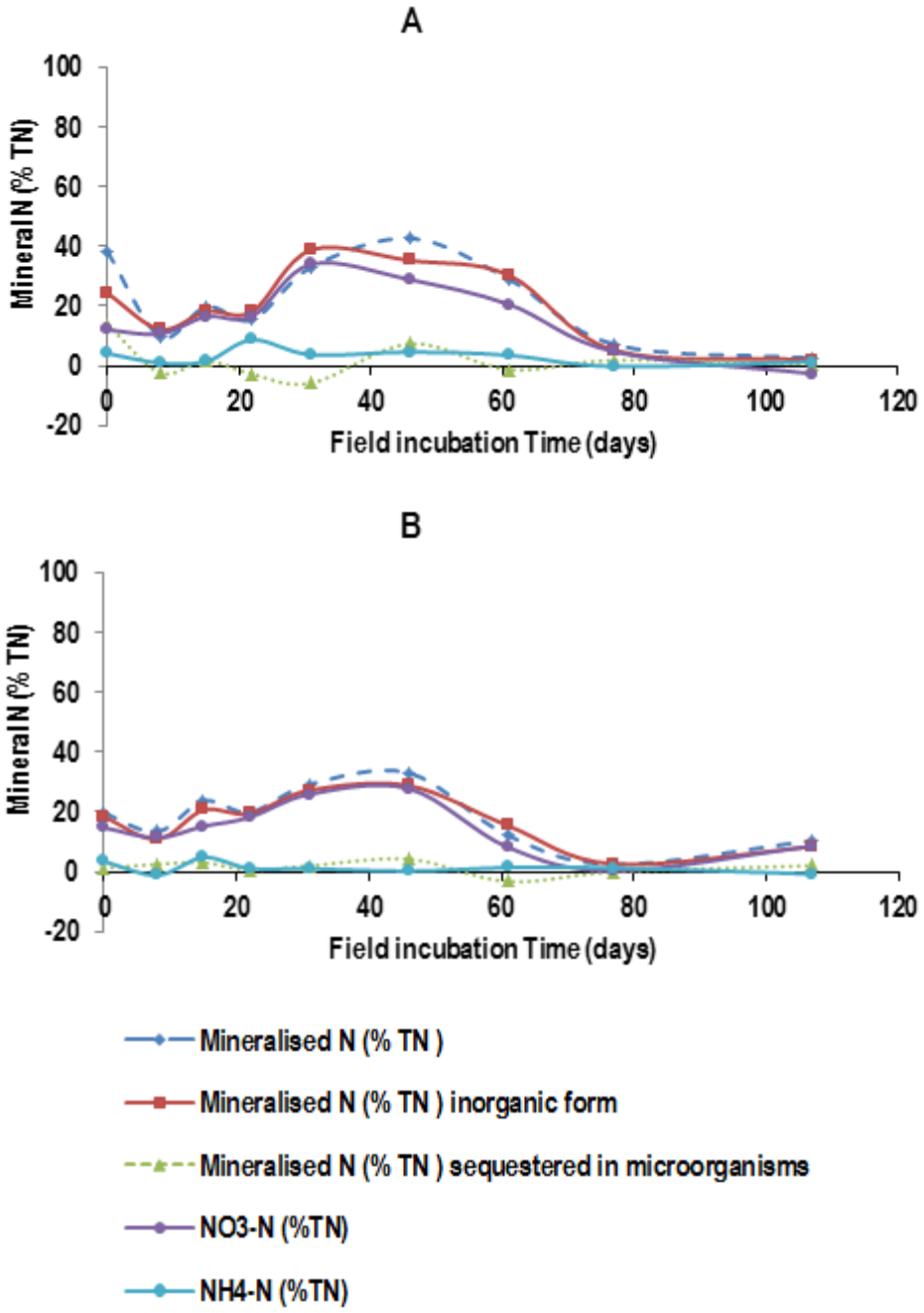

Figure 6.14. Mineral N (%TN added) from ADB-applied to the clay loam (A) and sandy loam soil (B)

The maximum N mineralised from ADB-amended the clay loam and sandy loam soil under field conditions without and with ryegrass are shown in Table 6.4. The amount of mineral N (% TN added) shows that there was higher amount of mineral N in the clay loam soil between without and with vegetation of the ryegrass, However, the

quantities of mineral N with vegetation of the ryegrass were slightly lower than the amount of total mineral N without ryegrass which is consistent with ANDB.

Table 6.4 Mineral N from ADB-amended two soil types under field incubation trail

ADB		Day of maximum mineralised N	%TN_{added} NH_4-N	%TN_{added} NO_3-N	%TN_{added} MBN	Mineralised N (% TN)	Organic-N mineralised (% org-N)
Clay loam soil	Field (no vegetation)	46	1 ± 0	49 ± 1	2 ± 1	51 ± 2	45 ± 1
	Field (vegetation)	46	5 ± 1	28 ± 2	8 ± 1	43 ± 1	35 ± 1
Sandy loam soil	Field (no vegetation)	31	-1 ± 1	47 ± 2	2 ± 0	48 ± 1	39 ± 1
	Field (vegetation)	46	0.2 ± 0.1	28 ± 1	4 ± 1	33 ± 1	22 ± 1

The maximum fraction of organic-N mineralised from ADB-amended the clay loam soil was 44 %, which is slightly higher than in the ADB amended sandy loam soil. This occurred on day 46.

On both the clay loam and sandy loam soils, the amount of Total mineralised N measured, was higher on the plots on which there was no vegetation.

6.3.2 Effect of biosolids type on mineral N

The ammonification process in ANDB-amended the clay loam and sandy loam soil was greater than in the ADB-amended both soil types. This was indicative of the long period in which ADB was stockpiled in which NH_4-N would have been lost.

In the amended clay loam soil, the production of NO_3-N from ANDB and ADB was similar on day 31. However, in comparison between two biosolids amended sandy loam soil, it can be seen that the NO_3-N produced from ADB was approximately 45 % greater than the corresponding NO_3-N recorded in ANDB.

6.3.3 Effect of soil type on mineral N

Comparing the two soil types, NO_3-N values produced on day 31 in the clay loam soil amended with ANDB and ADB were 63 % and 13 %, respectively, higher than

the corresponding NO_3-N level recorded in the less fertile sandy loam soil. Similarly, NO_3-N produced from unamended control clay loam soil was 8 % greater than the corresponding NO_3-N values recorded in the unamended sandy loam soil.

Fertile soils receiving biosolids provide adequate substrate (C) to soil microbes which increase soil microbes activity thereby increasing soil nitrification more than less soils fertility (Epstein, 2002, Rigby et al., 2009, Koenig et al., 2011, Lu et al., 2012, Cogger et al., 2013). In this case the clay loam soil had higher organic matter, water holding capacity and N content than the sandy loam soil (See Chapter 4, Table 4.7.).

6.3.4 Effect of biosolids and soil types on MBN and MBC

Comparing the two biosolids amendments on the clay loam soil, the concentration of MBN in ANDB was 34 % greater than ADB on day 1. This may be due to the quantity of organic matter contained in ANDB being higher than ADB (Chapter 4, Table 4.7). There was a significantly higher ($P < 0.01$) concentration of MBN recorded in ANDB whereas there was no significant difference ($P > 0.05$) the concentration of MBN detected in ADB compared to the unamended clay loam soil.

Of the two biosolids applied on the sandy loam soil, there was one and half times greater N immobilisation when applying ANDB than ADB by day 15. Analysis of variance showed that there was a substantially higher ($P < 0.001$) amount of MBN recorded in ANDB but there was no significant difference ($P > 0.05$) for ADB. The background of MBN concentration in the clay loam soil was higher than the corresponding of MBN concentration in the less fertile sandy loam soil (Chapter 4, Table 4.7).

The concentration of MBC observed from two biosolids-amended the clay loam soil was greater than the MBC concentration observed in the sandy loam soil.

Comparison in of % mineralisation in the field experiment without and with vegetation

In order to estimate the N mineralisation rates on different condition (without as shown in Chapter 5, and with ryegrass as shown in this chapter), the amount of mineral N added, plant available N the amount of organic-N immobilised and organic N mineralised are presented in Table 6.5.

Table 6.5 Mineral N of each treatment in laboratory and field incubation conditions

		Field incubation (No vegetation) [b]					Field incubation (vegetation) [b]			
		Mineral-N (%TN added)	PAN[a]	*MBN	Organic-N mineralised (%organic-N)		Mineral-N (%TN added)	PAN[a]	*MBN*	Organic-N mineralised (%organic-N)
Clay loam soil	Time (days)					Time (days)				
ANDB	46	56 ± 2	56 ± 2	-0.03 ± 1	54 ± 3	46	53 ± 1	50 ± 1	3 ± 1	50 ± 2
ADB	46	51 ± 2	50 ± 2	2 ± 1	45 ± 2	46	43 ± 1	35 ± 1	8 ± 1	35 ± 1
Sandy loam soil										
ANDB	31	51 ± 2	47 ± 2	4 ± 1	46 ± 2	15	48 ± 1	38 ± 1	9 ± 1	43 ± 1
ADB	31	48 ± 1	47 ± 1	2 ± 0	39 ± 1	46	33 ± 1	29 ± 1	4 ± 1	22 ± 1

[a]Plant available N

*****MBN after subtracting values from the unamended control plots values

[b]The values have been calculated using 15 cm soil depth of biosolids incorporation

Without vegetation, percentage mineralised organic-N was marginally higher for ANDB than ADB on the clay loam soil (54 ± 3 and 45 ± 2, respectively) and sandy loam soil (46 ± 2 and 39 ± 1, respectively).

With vegetation, percentage mineralised organic-N was higher for ANDB than ADB on the clay loam soil (50 ± 2 and 35 ± 1, respectively) and sandy loam soil (43 ± 1 and 22 ± 1, respectively).

6.4 Conclusion

The results obtained in this study concluded that there was an influence of types of biosolids on ammonification and nitrification process in both soil types. However, the effect was ~10 % greater in ANDB than ADB in two soil types.

The microbial biomass N and C concentrations were lower in two biosolids-amended two soil types with vegetation of the ryegrass than without vegetation of the ryegrass which is indicative of the competition between the ryegrass and soil microbes for the N availability from these materials. This competition is reflected in the amount of N mineralised as shown in Table 6.5. The quantities of organic-N mineralised in ANDB-amended the clay loam and sandy loam soil was greater than the amount of organic-N mineralised from ADB-amended the two soil types.

The results show that the N mineralisation obtained from the field experiment was closer to values cited in previous research. However, the fraction of organic-N mineralised from two biosolids was calculated and for more applicable of estimating the plant available N, biosolids and fertiliser added at an increasing rates and total N taken up by ryegrass was measured. The following chapter (7) describes the amount of organic-N mineralised from two biosolids compared to the fertiliser at different soil types.

7

7 An Assessment of the Victorian Guidelines for Land application of Biosolids based on Plant Available Nitrogen

7.1 Introduction

In this chapter the plant available N is quantified relative to urea, for two different types of biosolids, applied to two soil types. It also studies the effect of soil type and biosolids type on plant available N under field conditions.

Most of the N in biosolids is in the organic form, which is not immediately available to plants. However, over time, the organic N is converted to mineral N (NO_3-N and NH_4-N) via decomposition by microorganisms (mineralisation). The mineralised N is then available to the crop. The best application rates for biosolids will provide just the right amount of nutrient required by the plant, while minimising the potential for environmental risk through losses of N via ammonia (NH_3-N) volatilisation, nitrate (NO_3-N) leaching, or nitrous oxide (N_2O) greenhouse gas emissions. On the other hand, if there is insufficient N available, crop production will be compromised (Cogger et al., 1999, Smith and Durham, 2002, Morris et al., 2003, Cogger et al., 2004, Corrêa(1), 2004). Therefore, predicting N availability is a factor in applying a safe and efficient amount of biosolids (N, P, K and S) without potential environmental risk.

In general, the application rate for biosolids is calculated by the 'Nitrogen Limited Biosolids Application Rate' (NLBAR). The NLBAR concept ensures that the biosolids applied supply enough PAN to meet the crop requirement as describe in chapter 1. In this method, it is assumed that 15 % of the organic N from anaerobically digested biosolids and 25 % of the organic N from aerobically digested biosolids, becomes

available for the crop in the first year following the application (EPA, VIC ,2004). However, these mineralisation rates are based upon overseas data, not on data obtained in Australia with Australian soils and climatic conditions.

Nevertheless, a number of field studies have been conducted in Australia on the mineralisation rates of biosolids in Australia in South East Queensland (Pu et al., 2008), New South Wales (Eldridge et al., 2008) and Western Australia (Rigby, 2010).

The work of Pu *et al.,* (2008) was conducted under the Australian National Biosolids Research Program (NBRP) which was established by the CSIRO in 2002, and mainly focussed on the solubility and bioavailability of metals (Cd, Cu, and Zn) from biosolids applied to land for beneficial use. Pu *et al.,* (2008) determined the fate of N supplied from anaerobically and aerobically produced biosolids applied to a clay loam soil in the sub-tropical climate of South-East Queensland, on a forage sorghum production system. The organic N fraction found to be available for the crop in the first year following application, ranged from 43 to 59 % of the applied organic N. This is significantly greater than the values assumed by the Victorian and NSW EPA for the mineralisation of organic N in biosolids (EPA, NSW, 1997, EPA, VIC ,2004). It is obvious that the assumed mineralisation rates for N used in the New South Wales and Victorian Guidelines are not applicable for Queensland conditions.

Eldridge *et al.,* (2008) also investigated N mineralization of organic N from biosolids. Their experiment was conducted in New South Wales using both field and laboratory incubation experiments. The fractions of organic N mineralised in their 12-month field incubation study using granulated biosolids (12, 24, and 48 dry t ha^{-1}) and dewatered biosolids (22 dry t ha^{-1}) with silty clay loam in texture were estimated to be 54 %, 48 %, 45 %, and 53 %, respectively under a temperate climate. It would appear that the values used for organic N mineralisation to determine the application rate for biosolids are not applicable in NSW either.

Rigby *et al.,* (2010) investigated the PAN from three biosolids types applied to an acidic sandy soil under a Mediterranean type climate in Western Australia. The findings showed that the PAN of biosolids relative to urea (N) was dependent upon the treatment process of the biosolids, with 65.1 %, 63.9 % and 39.4 % PAN value for lime amended biosolids, aluminium dosed biosolids and dewatered biosolids respectively. The amount of organic N that became available in the first season was

2 - 3 times greater than the current estimate value of 20 % used to compute the loading rate for biosolids in Western Australia.

Based on the Australian field experiments cited above, there is clear evidence that the mineralization of organic N in soils amended with biosolids, do not correspond to values obtained in other countries. This is not surprising since mineralisation depends on several environmental and climatic conditions which include soil type, moisture, pH, temperature, C: N ratio and type of biosolids (Tester et al., 1977, Smith et al., 1998a, Smith et al., 1998c, Honeycutt et al., 1991, Sierra et al., 2001a, Rahman and Rashid, 2002, Morris et al., 2003, Wennman and Kätterer, 2006, Pu et al., 2012). Accordingly, there is a significant research gap regarding accurate quantification of PAN from different biosolids applied to different soil types under various climatic conditions.

The organic N availability from biosolids, or other organic wastes, with or without a crop, can be estimated by calculation of an N material balance (Mamo et al., 1999, Torstensson and Aronsson, 2000, Pu et al., 2008). This process is tedious as it normally involves the measurement of microbially sequestered N, plant uptake, NH_3, N_2O, N_2 organic N in the soil and any leached N.

An alternative method, used in this study, is to estimate the PAN content of biosolids under field or glasshouse conditions by comparison with a crop response (in terms of yield or N uptake) to the crop response to an N fertiliser (Barbarick and Ippolito, 2000, Smith et al., 2002b, Morris et al., 2003, O'Connor et al., 2004, Pritchard, 2005a, Barbarick and Ippolito, 2007, Pritchard et al., 2010). This is generally achieved by generating a calibration curve of crop response versus 5 - 6 rates of inorganic fertiliser N, ensuring that only the nutrient under investigation is limiting. All other nutrients are supplied according to crop requirements. This method assumes that the generation of available N from the N fertiliser is 100 % efficient, and that the plant will take up the mineral N from the fertiliser in the same way it does from the mineralised N from the biosolids. Since the environmental conditions are the same for the crops treated with fertiliser and those treated with biosolids, the pathways for losses (leaching, volatilisation and immobilisation by microorganisms) of the mineralised N will also be the same. However, if the rate of mineralisation of N from the fertiliser is faster than the rate of mineralisation of the biosolids, then the environmental conditions and stage of plant growth will vary to some degree.

The slope of the calibration for the plots with added biosolids over the slope of the calibration curve for the plots with added urea can be used to determine the amount of mineralisable N, provided the calibration curves are in the linear range (in other words, the plants are receiving less than their required N).

The logic behind the calibration method is as follows: we can say that if the plants take up A % of TN from urea, they will take up A % from the mineralised fraction of N from the biosolids (because the environmental conditions are the same and the mineralised N is in the same form). In the case of biosolids, this will include both inorganic and mineralised organic N.

If B % of the total N from the biosolids is taken up into the plants, then B % of TN from biosolids = A % of the mineralised N from the biosolids

Hence the fraction of mineralised N from the biosolids = (B × TN from biosolids) / A

B / A is the ratio of the two calibration slopes.

Quantifying the fertiliser N equivalent relative to various applications of biosolids is essential when assessing agronomic rates for different crops. This experiment was conducted to provide quantitative information on N availability for plants using different types of biosolids.

The overall aim was to assess the biosolids guidelines for land application for agricultural use. Ultimately, the desired outcome is to optimise the application rate such that the nutrient requirements of the plants are met, while minimising nutrient losses to the environment, which can occur if biosolids are applied in excess.

The specific aims of this experiment was to quantify the amount of PAN in two different biosolids types used to amend two types of soil, a clay loam and a sandy loam, under different field conditions. In addition, the effect of soil type and type of biosolids on the N availability was investigated.

The results of these experiments will contribute relevant information to improve the Victorian guidelines for land application of biosolids.

7.2 Establishing Nitrogen Calibration plots

In order to quantify the plant available N value of biosolids under filed conditions, a systematic design (Smith and Hadley, 1988, Smith et al., 2002b, Morris et al., 2003)

was used with three treatment types (two biosolids types and urea applied based on TN). The amount of urea added was from lower than the crop requirement (250 kg N ha^{-1}) to just above it. The amount of the biosolids required to provide the equivalent amount of N to the amount supplied by urea was determined using the current EPA guidelines for NLBAR (EPA, VIC ,2004).

The size of this experiment was 38 m×11.5 m .The land was partitioned into three main plots within a size 1.5 m× 12 m; each plot was divided into 6 subplots with a size 1.5 m × 2 m. The design (a randomized complete blocked design, RCBD) includes 3 replicates (Blocks) x 3 treatment types x 6 application rates (Figure 7.1).

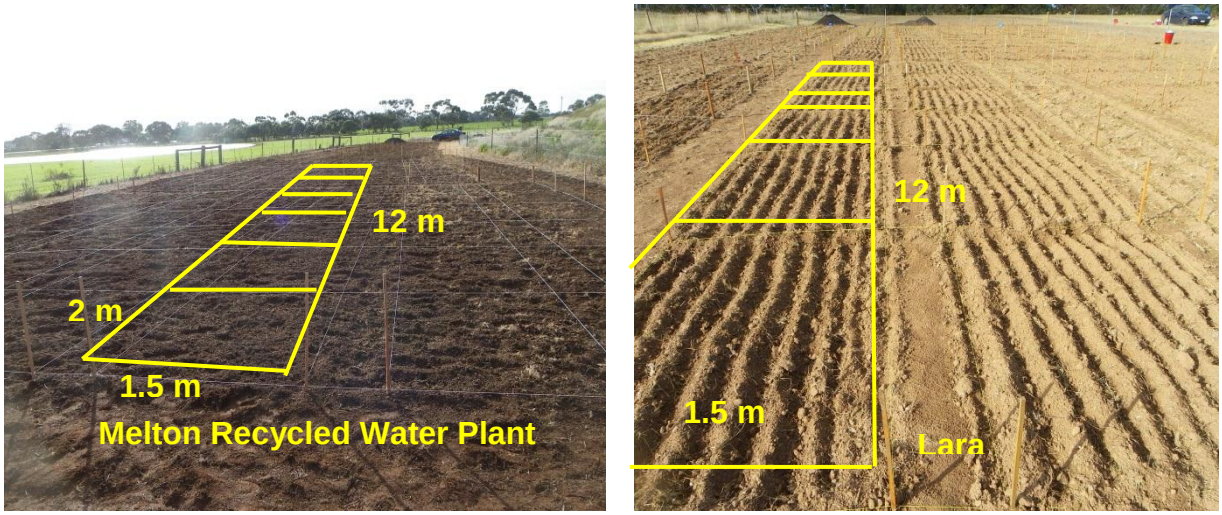

Figure 7.1. The partitioning into main plot and then subplot in both sites

The treatments for each site were: 2 biosolids types x 1 nutrient (N) + 1 fertiliser types (N). The biosolids and fertiliser application rates were randomised within each block, and the application rates were also randomized across the gradient indicated by the arrows in Figure 7.2.

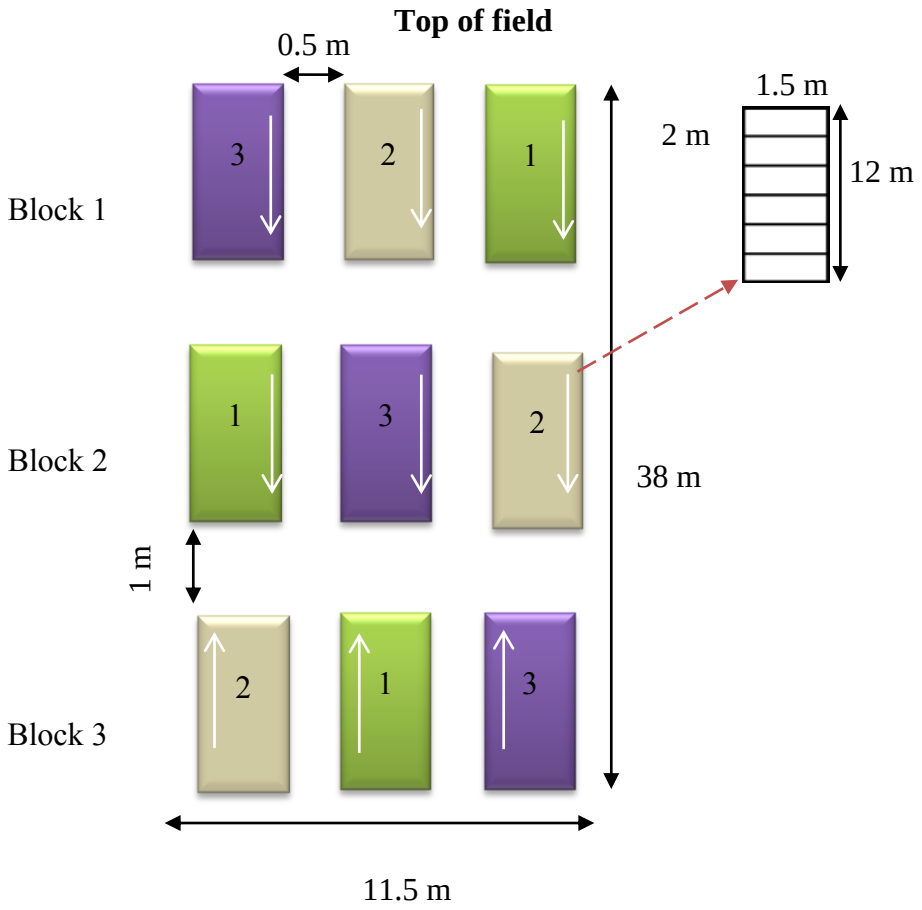

Figure 7.2. The systematic design where treatments types (two biosolids types and urea) and sub treatments (application rate) were arranged in a randomized complete block design (RCBD) of the fertiliser calibration plot, The numbers 1,2 and 3 refer to fertilizer treatments (Urea - 46% N), o biosolids from MRWP and , biosolids from BW applied based on N respectively. The arrows indicate the direction of application of biosolids and urea.

Urea was applied base on the maximum requirement or the recommended rate for perennial ryegrass which was of 280 kg N ha^{-1}. The plots have been established, with increasing the amount of N. The application rates are presented in (Table 7.1).

Table 7.1 Nitrogen fertiliser application rates (Urea - 46% N)

Sub-treatments	kg N/ha	kg urea/ha	kg urea/3m^2	g urea/3m^2	g N/3m^2
1	0	0	0	0	0
2	60	130	0.039	39.1	18
3	120	261	0.078	78.3	36
4	180	391	0.117	117	54
5	240	522	0.157	157	72
6	280	609	0.183	183	84

Biosolids samples were analysed for total N and then the application rate to provide similar amount of N to that being applied by fertiliser was calculated. However, since not all of the N will be available to the crop. The application range was extended more than the total N fertilizer. Biosolids application rates are shown in Table 7.2 and Table 7.3.

Table 7.2 Biosolids application rates based on Nitrogen (Biosolids from MRWP), 22.5 kg N/tonne

Sub-treatments	Kg N/ha	t ds/ha	kg ds/ha	kg ds/3m^2	g ds/3m^2	g N/3m^2
1	0	0	0	0	0	0
2	68	3.02	3022	0.91	907	20.4
3	136	6.04	6044	1.81	1813	40.8
4	204	9.07	9067	2.72	2720	61.2
5	340	15.1	15111	4.53	4533	102
6	510	22.7	22667	6.80	6800	153

t ds = tonnes dry solids

.

Table 7.3 Biosolids Nitrogen application rates (Biosolids from BW), 20.27 kg N/tonne

Sub-treatments	kg N/ha	t ds/ha	kg ds/ha	kg ds/3m^2	g ds/3m^2	g N/3m^2
1	0	0	0	0	0	0
2	68	3.35	3355	1.01	1006	20.4
3	136	6.71	6709	2.01	2013	40.8
4	204	10.1	10064	3.02	3019	61.2
5	340	16.8	16774	5.03	5032	102
6	510	25.2	25160	7.55	7548	153

To provide a basal dressing for the N fertiliser treated calibration plots, a complete trace elements fertilizer containing 0.5 % Cu, 1.1 % Zn, 3.1 % Mn, 12 % Fe, 3.5 % Ca, 2 % Mg, 0.1 % Bo, 11.5 % S and 0.04 % Mo was applied at rate of 100 kg ha^{-1} on N fertilizer treated plot. Muriate of Potash (41 % K) at rate 30 kg ha^{-1} was also applied to serve as sources of macronutrients. Superphosphate was applied at a rate 50 P ha^{-1} to the N fertilizer calibration plot.

Biosoilds and fertilizer were weighed into plastic containers using a top load weighing balance and spread by hand into the topsoil. They were immediately incorporated using a rake to a 10 cm soil depth (Figure 7.3)

Figure 7.3. Application of biosoilds and fertiliser, distributing the materials on the top of soil and then incorporating at 15 cm soil depth

Perennial ryegrass was sown to a 1 cm depth at a rate of 200 kg seed / ha which was equivalent to 360 g / 18 m^2 per treatment, plant space was divided at 20 cm rows each plot has been used 10 rows on each plot, on the 7th and 12th of June 2011 at LA and MRWP sites “respectively” and then it cover by soil immediately (Figure 7.4).

Figure 7.4. Perennial ryegrass was sown at a rate of 200 kg seed / ha

Perennial ryegrass samples were harvested from calibration plot on 22nd and 23rd of September 2011 at MRWP and 27 October 2011 at LA site. The samples were harvested from 1 m × 1 m quadrants from the centre of each of the subplots, leaving 2 cm above the ground. The samples were transferred into paper bags and transported to the laboratory where fresh biomass was recorded. There was a time delay of between 3 - 6 hours from the time of harvest to the time of measurement (Figure 7.5). Totat N was measured as decribed on chapter 3 (See 3.6).

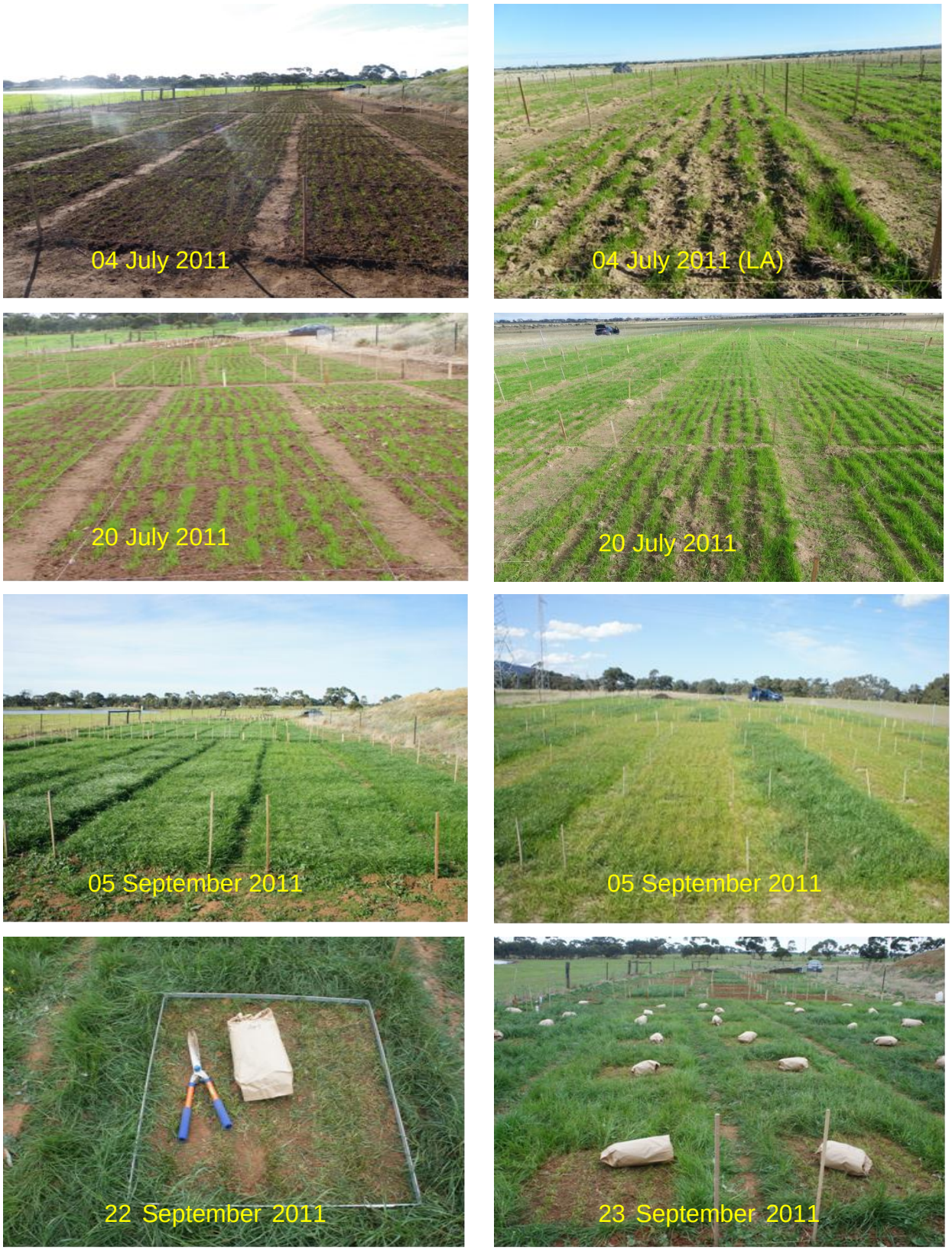

Figure 7.5. Growth phase and harvesting ryegrass at two sites

7.3 Results and Discussion

7.3.1 The response of ryegrass to the application of biosolids and urea

The response of ryegrass to the application of biosolids applied based on nutrients and one fertilisers (urea) applied on two soil types are shown in

Figure 7.6 – Figure 7.8.

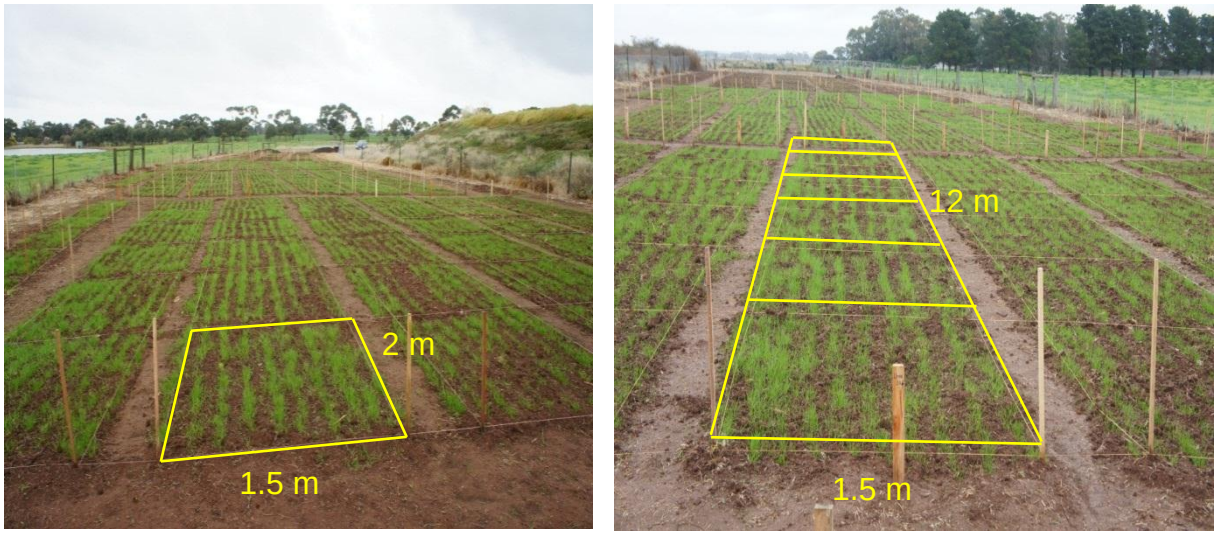

Figure 7.6. Ryegrass response to biosolids and fertiliser application rates applied at increasing rates of N on the clay loam soil at the Melton Recycled Water Plant (23 July 2011, week 4 after sowing)

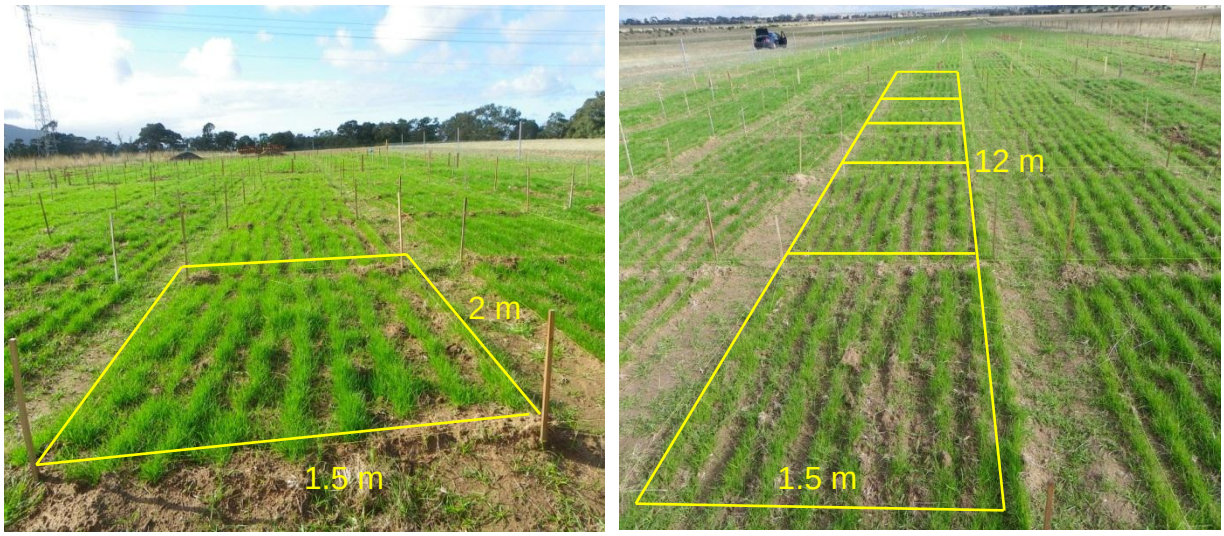

Figure 7.7. Response of ryegrass to the application rates of biosolids and fertiliser applied on the sandy loam soil at Lara (23 July 2011, week 4 after sowing)

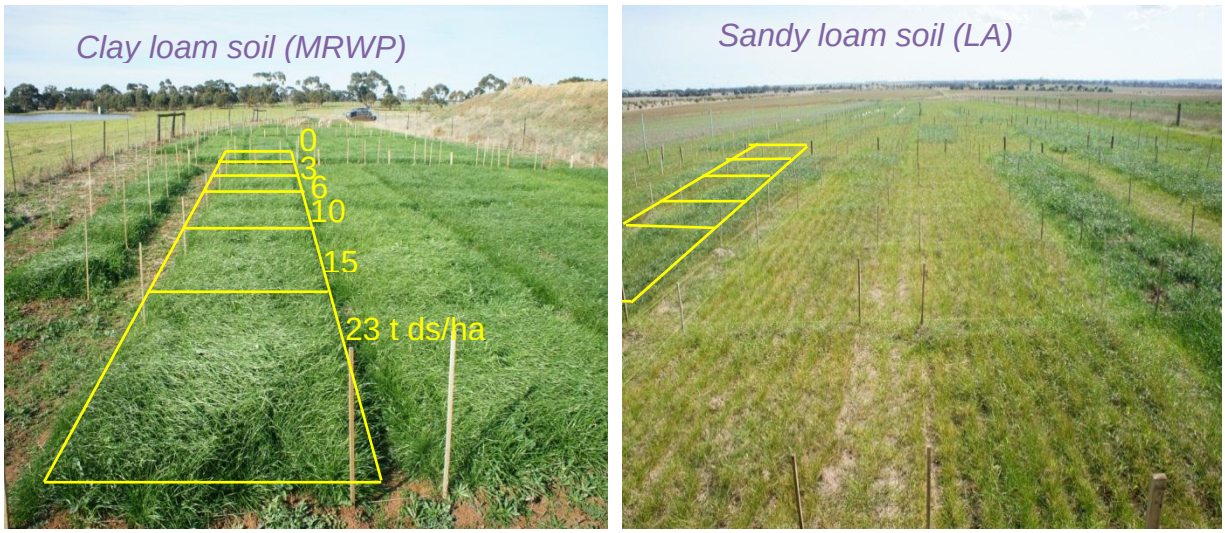

Figure 7.8. Ryegrass response to the application rates of biosolids (t ds ha^{-1}) and fertiliser (kg N ha^{-1}) at different soil types (05 Sep 2011) after 120 days sowing plant

These photographs show the growth of the crop at various stages during the growing period. The growth of ryegrass on the clay loam soil was clearly more vigorous than on the sandy soil. This could be due to a number of factors determine the capacity of a soil to produce plants (CEC, fertility , available water , aeration, and so on): the sandy loam soil had less organic carbon and had a lower moisture holding capacity as indicted in Chapter 4 (Table 4.7). This is consistent with work of Cogger *et al.,* (1999), and Muchovej and Rechcigl (1998). Figure 7.9 shows the TN uptake by

ryegrass grown in the two soil types studied here when biosolids and fertiliser are applied. As expected, there was a significant correlation between the amount of N applied and crop response (*P* value = 0.05, R^2= 77.1%) (Table 7.4). Also, as expected, the crop response to the application of urea was greater than the application of N from biosolids due to the N from urea being more readily mineralised than the N from biosolids (Rigby et al., 2010).

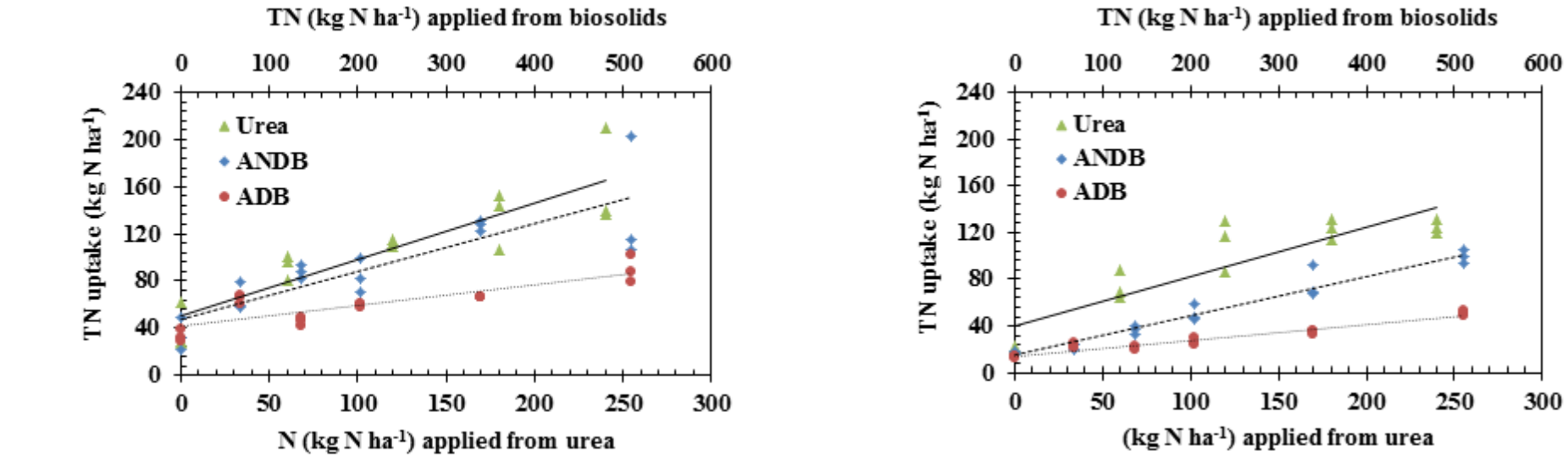

Figure 7.9. Effect of applying various rates of urea, anaerobically digested biosolids (ANDB) and aerobically digested biosoilds (ADB) on plant uptake of TN (kg N ha^{-1}) by ryegrass on (A) clay loam soil and (B) sandy loam soil

To determine the amount of urea equivalent N in biosolids for ryegrass, the plant uptake of TN per mass of dry matter was plotted against the application rate of biosolids and urea (Section 7.1). From these measurements, the ratio of the slope of the biosolids application (kg N ha^{-1}) curve to the slope of curve for the application of urea (kg N ha^{-1}) was calculated. Only the linear portion of the response was used as this represents the portion of the graph when the plant was undersupplied with N and therefore is most likely to utilise all that is available (Figure 7.10). In the case of urea, the response of ryegrass reached a maximum before the highest application rate of 280 kg N ha^{-1}.

From the slope of Figure 7.9, the calculated N fertiliser equivalency value for ANDB was greater than ADB on clay loam and sandy loam soil when TN uptake was used as an indicator of crop response (Figure 7.9). The results show that the N mineralisation occurred rapidly at earlier stages after biosolids application to the clay loam soil in comparison to the sandy loam soil because of the higher fertility of clay loam soil due to higher microorganism population. Microorganisms breakdown the organic matter and convert the N to mineral forms which can be easily taken up by the crop.

The ryegrass response was also measured in terms of fresh and dry biomass and graphed against the applied biosolids N and fertiliser N as shown in Figure 7.10.

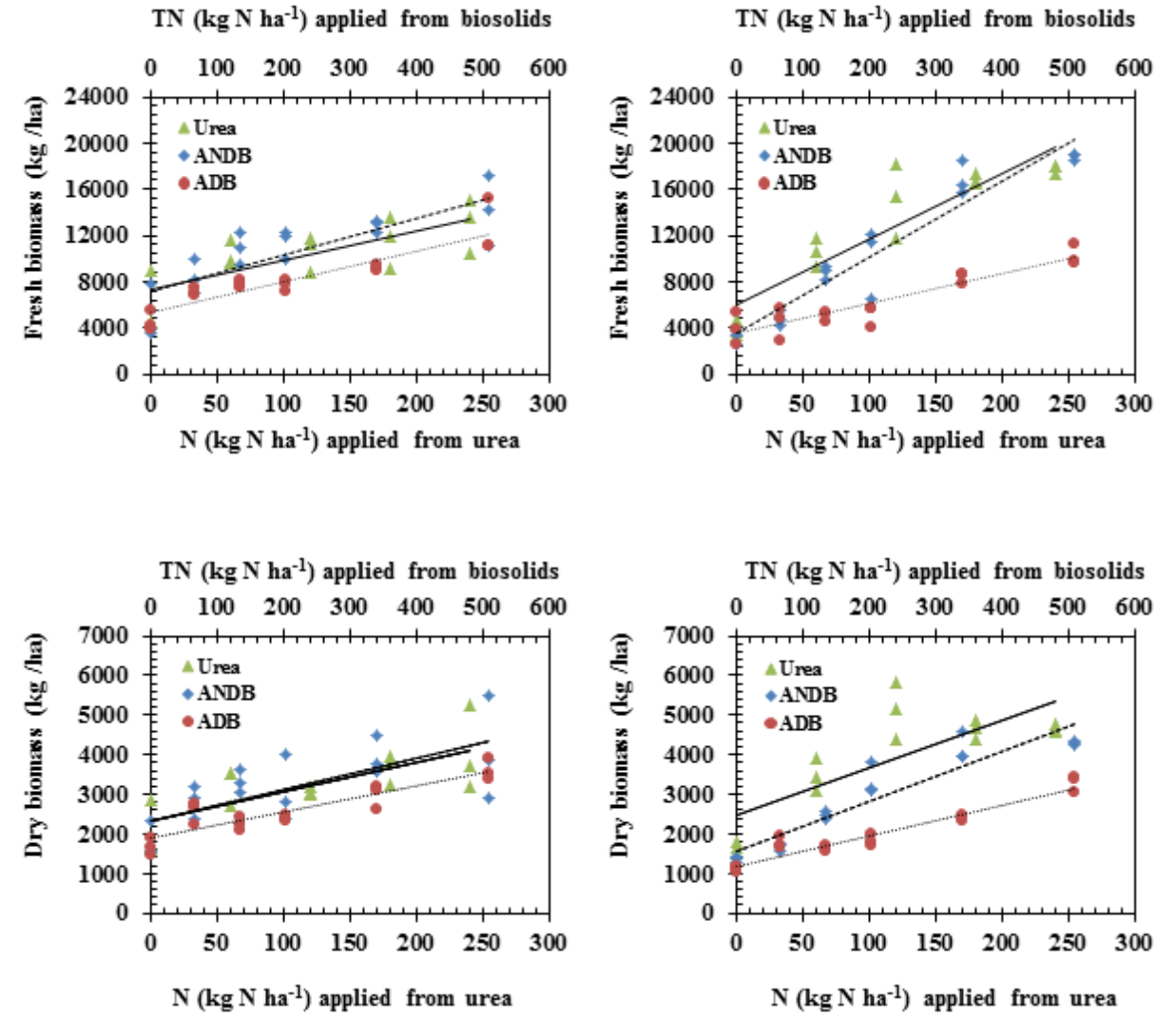

Figure 7.10. Effect of applying various rates of urea, anaerobically digested biosolids (ANDB) and aerobically digested biosoilds (ADB) on fresh and dry biomass (kg/ ha) by ryegrass on (A) clay loam soil and (B) sandy loam soil

Fresh and dry yield and N uptake in relation to TN applied from biosolids and N fertiliser were statistically analysed using linear regression and compared to yield data from mineral N calibration plots. The results were fitted to a linear model form $y = a + b\,x$ where y is the fresh, dry biomass or N uptake (kg ha^{-1} fresh or dry matter or TN uptake kg N ha^{-1}) and x is the biosolids application rate based on TN (kg N ha^{-1}) or the application of urea (kg N ha^{-1}).

The effect of urea on ryegrass on the clay loam soil was significant (P = 0.001) (See Table 7.4) when TN uptake and fresh biomass are the parameters considered. There was a significant effect of block design (P = 0.049, 0.020) on the TN uptake and fresh biomass respectively as well. This indicates that there was a possible unknown source of N in one of blocks.

Analysing the effect of applied ANDB to clay loam soil using fresh biomass as indictor showed significant impact of the block (P = 0.026). The relationship between rate of N applied and ryegrass response when measured as fresh and dry yield was statistically significant (P = 0.001).

Table 7.4 Summary results of using Minitab 16 software for regression and two-way ANOVA for all treatments

Site	Treatments	Parameters	Two way ANOVA		Regression			
			P-value	Block effect	Equation	Standard Error	*P*-value	R^2 (%)
Melton Recycled Water Plant (Surbiton Park)	Urea	Fresh biomass	0.003	0.020*	y= 7253+25.5x	6.28	0.001	52.5
		Dry biomass	0.011	0.073	y= 2341+7.42x	1.78	0.002	51.2
		TN uptake	0.001	0.049*	y= 50.6+0.479x	0.06	0.001	77.1
	ANDB	Fresh biomass	0.001	0.026*	y= 7148+15.9x	17.9	0.001	63.8
		Dry biomass	0.024	0.263	y= 2345+3.95x	0.99	0.001	46.5
		TN uptake	0.003	0.398	y= 47.6+0.202x	0.03	0.001	69.4
	ADB	Fresh biomass	0.001	0.436	y= 5335+13.4x	11.8	0.001	80.6
		Dry biomass	0.001	0.405	y= 1893+3.27x	0.42	0.001	77.2
		TN uptake	0.001	0.712	y= 41.3+0.0886x	0.01	0.001	68.4
Lara	Urea	Fresh biomass	0.001	0.601	y= 6063+56.3x	6.97	0.001	82.2
		Dry biomass	0.001	0.682	y= 2483+11.9x	2.63	0.001	58.1
		TN uptake	0.001	0.140	y= 39.5+0.427x	0.05	0.001	78.4
	ANDB	Fresh biomass	0.001	0.365	y= 3587+32.7x	2.46	0.001	91.1
		Dry biomass	0.001	0.775	y= 1566+6.30x	0.61	0.001	86
		TN uptake	0.001	0.908	y= 15.0+0.169x	0.009	0.001	95.3
	ADB	Fresh biomass	0.001	0.010*	y= 3472+13.0x	1.39	0.001	83.6
		Dry biomass	0.001	0.673	y= 1198+3.84x	0.28	0.001	91.6
		TN uptake	0.001	0.230	y= 14.0+0.0671x	0.004	0.001	93.6

Treatments: Urea, anaerobically dewatered digested biosolids and aerobically dewatered digested biosolids. *There is a significant effect of block design

The effect of urea application on ryegrass in clay loam soil was significant (P = 0.001) (Table 7.4) when TN uptake and fresh biomass are the apartments considered.

A statistically significant block effect was observed for urea and ANDB treatments as measured by fresh biomass in clay loam soil (Table 7.4). This could be simply due to one of the blocks being a little wetter than the others. There was also significant block effect on TN uptake by crop from urea applied into clay loam soil. This may be due to residual N from an unknown source.

Data obtained for fresh biomass using ADB applied in the sandy loam soil showed a significant block effect (P = 0.010), which may be due to the slight slope of land used in this study.

Statistical analysis (Minitabe 16 software) used in this study to examine the level of impact of the biosolids treatments of the two soil types as well as the effect of randomise block design compared to control plots on the availability of TN for ryegrass (Table 7.4).

A summary of the statistical analysis, linear regression coefficient, standard error (SE) and R^2 values are provided in Table 7.5. By comparing regression coefficient of biosolids with linear regression coefficient of urea as described in section 7.3.1., PAN values were estimated for two biosolids types that demonstrated a significant relationship between the rate of biosolids application and ryegrass response at probability level of P = 0.001 (Table 7.4)

Table 7.5 Linear regression coefficients (Slope), standard error and R^2 summarised for each of the biosolids and urea at Melton Recycled Water Plant and Lara field experimental sites

	Fresh yield			DM[1] yield			N Uptake		
	Slope	SE (%)*	R^2	Slope	SE*	R^2	Slope	SE*	R^2
Clay loam soil									
Urea	25.5	24	0.55	7.41	25	0.54	0.47	12	0.78
ANDB	15.8	113	0.65	3.94	25	0.79	0.20	15	0.71
ADB	13.3	89	0.81	3.27	13	0.78	0.08	12	0.70
Sandy loam soil									
Urea	56.5	12	0.83	11.8	20	0.61	0.42	11	0.79
ANDB	32.6	8	0.91	6.29	9	0.86	0.16	5	0.95
ADB	13.0	10	0.84	3.84	7	0.92	0.06	6	0.93

[1] Dry yields

* Standard error (%) of slope was calculated using Minitab (16) software

It can be seen from Table 7.5 that the rate of uptake of N from urea is between 42 and 47 % which is typical for urea (Cogger et al., 2004) and is consistent (41 %) with work of Rigby *et al.,* (2010), with the lower value being obtained on the sandy loam soil at LA. The two biosolids had a much lower percentage N uptake than urea, with ADB being about half of that of ANDB. In both cases, the percentage uptake was slightly lower on the sandy soil (as it was with urea).

A major factor affecting the N availability from biosolids treated soil is soil type, where the soil with higher content of clay is an indicator of higher fertility and higher amount of organic matter (Chae and Tabatabai, 1986a, Jha et al., 1996b, Silva et al., 2005b, Corrêa et al., 2006, Rigby et al., 2009). This is supported by the findings from a field experiment conducted by Rigby *et al.,* (2009) which showed that the initial mineralisation and nitrification of biosolids applied to a silt clay soil which contained a large amount of organic matter were higher than those in a sandy loam with less organic matter.

Table 7.6 Test of significance for soil type for each treatment using the Student t test

Treatments		TN uptake	Fresh biomass	Dry biomass
Urea	P-value	0.244*	0.092*	0.048
ANDB		0.003	0.981*	0.441*
ADB		0.000	0.030	0.016

* Not significant ($P > 0.05$)

A significant effect was observed for the ADB application in the two soil type's studies here, where soils have different texture, fertility and organic matter as described in Table 7.6, which consequence on the amount of mineralisable N from different biosolids used in this experiment. Furthermore, there was a significant effect of the urea as source of N when the dry biomass used as a test of crop response. There was a significant effect of the ANDB on the measured TN uptake in each soil type (Table 7.6).

Many studies have investigated the effect of biosolid types on mineralisable N fraction in aerobically and anaerobically digested biosolids (Parker and Sommers, 1983, Gilmour and Skinner, 1999, Barbarick and Ippolito, 2000, Wang et al., 2003b, Barbarick and Ippolito, 2007, Pu et al., 2012, Cogger et al., 2013). The higher variability observed in mineralisable N values is to some extent related to the method employed to dewater the biosolids or the method or duration of storage (Gilmour et al., 2003). Rigby *et al.* (2010) reported that the N availability from biosolids relative to fertiliser was influenced by the biosolids treatment process.

Table 7.7 Examination of significance for two biosolids treatments for each soil types using the Student t test

Soil type		TN uptake	Fresh biomass	Dry biomass
Clay loam soil	P-value	0.009*	0.028	0.041
Sandy loam soil		0.007	0.009	0.011

* Significant effect ($P < 0.05$)

Table 7.7 lists the t-test parameters used to examine the effect of biosolids types on the two soil types studies here. There is a significant impact of biosolids, ANDB and

ADB, on the clay loam and the sandy loam soil, which reflects the differences in physiochemical properties of the biosolids types used in this experiment (See Chapter 4, Table 4.7).

7.3.2 Quantifying the PAN values of biosolids relative to urea

The N fertiliser equivalent evaluation results of the two biosolids applied on two different soil types are presented in Table 7.8. The ratio of the slope for the biosolids over the slope for urea represents the fraction of mineralised N (this includes both the inorganic and organic fraction as discussed in the introduction).

Quantification of PAN values relative to inorganic fertiliser were calculated and expressed as the ratio of plant available N corresponding to each parameter listed following the method of Barbarick and Ippolito (2000) in Table 7.8.

Table 7.8 Ratio of the regression slopes of the plant nitrogen uptake from biosolids versus urea

Soil types	**Parameters measured**	**Biosolids types**	
		ANDB	ADB
		Plant available N (%)	
Clay loam soil	Fresh yield	62 ± 19*	52 ± 14
	Dry yield	53 ±19	44 ± 7
	N Uptake	42 ± 9	19 ± 4
Sandy loam soil	Fresh yield	58 ± 8	23 ± 4
	Dry yield	53 ± 13	32 ± 8
	N Uptake	40 ± 6	16 ± 2

*Absolute uncertainty was calculated using the method described by Harris (2003),

$\%e = \sqrt{(\%e_1)^2 + (\%e_2)^2}$ Where % e i = standard error of slope i.

The estimated plant available N (PAN) value relative to urea on the clay loam soil for ANDB (62 %) was higher than for ADB (52 %) using fresh yield as an indicator of crop response. A similar trend was observed for ANDB and ADB applied to the sandy loam soil. The values using fresh biomass were elevated above those for dry biomass and TN and this was also, observed by Rigby *et al.,* (2010).

From a mathematical perspective the reason is that you get relatively more biomass than N when applying biosolids. This could be a real phenomenon, as the biosolids add other nutrients and minerals which enhance growth. Of course, this should be the same relationship for the dry biomass. The fact that it isn't, may be because the fresh biomass grown on the biosolids retained moisture better.

The PAN was higher when dry biomass was used as an indicator of crop response to the application of ANDB compared to the ADB to clay loam and sandy loam soil.

From the results for N uptake, it can be seen that the calculated amount of % mineralised N for ANDB is the same on both soil types within (~40 %) experimental error. The determined mineralisable N proportion was double for ANDB, 42 % and 40 % compared to 19 % and 16 % organic N of ADB for both soils. The values for ADB are also the same within (~17 %) experimental error for both soils (Table 7.8). It is interesting to note that the PAN (as the N fertiliser equivalent) for N uptake determined was lower than the PAN calculated from fresh and dry biomass in two soil types. As suggested by Kiemnec *et al*. (1987), N uptake may have been a more sensitive indicator of N availability than yield. In addition, other nutrients sources were applied and the contributed to increased crop growth in biosolids treatments relative to the mineral N control, then TN uptake would, of course, increase to some extent as a function of increased crop productions.

The findings from this study show that the N equivalency of ANDB and ADB in TN uptake determined was slightly higher in clay loam soil in comparison to sandy loam soil.

Absolute uncertainty was calculated using the method described by Harris (2003) using the following equation:

$$\%e = \sqrt{(\%e_1)^2 + (\%e_2)^2}$$ *Eqn.12*

Where % e i = standard error of slope i.

In this study, it has been assumed that all the urea was mineralised and that the entire mineralisable organic N in the biosolids was mineralised over the course of the field experiment, under the conditions of low application rates, warm temperatures and maximum plant uptake efficiency. This is consistent with findings shown by Pu *et al.,* (2008) and Rigby *et al.,* (2010).

The ratios for dry and fresh matter are higher than TN uptake. Using fresh matter requires immediate measurement following harvest and constant environmental conditions. This was not possible in our field experiment as it took close to a full day to harvest the ryegrass and there was further delay in weighing the material. Using dry matter assumes a constant relationship between the mass of dry matter and the N content of the material. However, the N content of the dry plant material increased with increasing application rates as shown in Figure 7.11. Consequently, in this case, it is not appropriate to use dry matter as a surrogate for N uptake. The biosolids also provide other nutrients in addition to N which may contribute to increased crop growth.

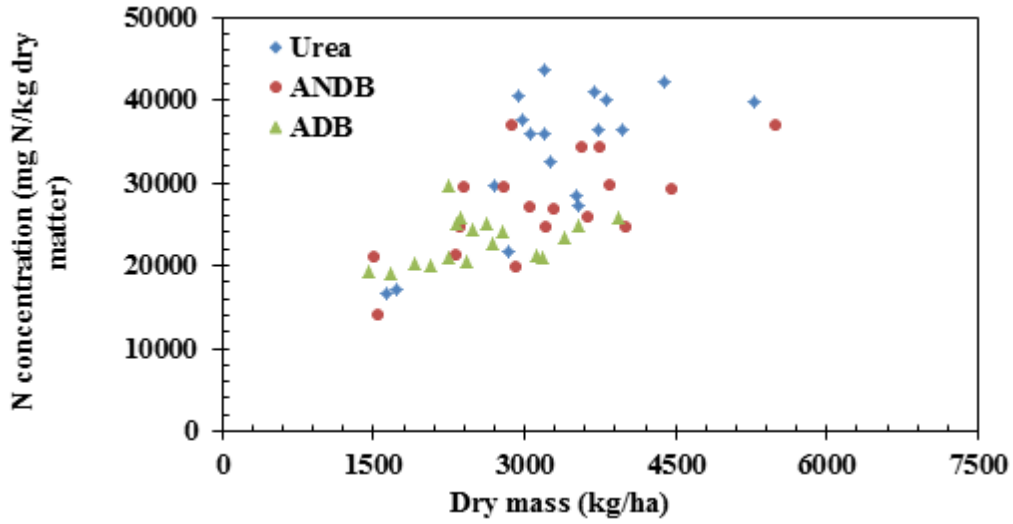

Figure 7.11. Relationship between plant N content and dry mass produced per hectare

A basal dressing of complete trace elements fertiliser was applied at rate of 100 kg ha^{-1} on the N fertiliser treated plots. Superphosphate (P-50 kg P ha^{-1}) and Muriate of Potash (41 % K) at rate 30 kg K ha^{-1} were supplied to the N fertiliser calibration plots to ensure that the soil was not deficient in any nutrient other than N. The biosolids treatments did not receive this basal dressing or superphosphate, because it contained enough micronutrients and macronutrients to satisfy the crop requirements even at the lower application rates (Table 7.9).

Table 7.9 The application rates of nutrients added from biosolid

Nutrients applied from biosolids (kg /ha)											
N	P	K	Cu	Zn	Mn	Fe	Ca	Mg	Al	S	Na
0	0	0	0	0	0	0	0	0	0	0	0
68	39	60	75	172	111	223	65	39	347	1882	32
136	79	119	150	344	223	447	129	77	695	3764	63
204	118	199	225	517	335	1043	194	116	1043	5652	95
340	196	299	347	861	557	1736	323	193	1736	9409	159
510	295	458	563	1294	838	2610	486	291	2610	14144	238

Table 7.9 shows the amount of P applied from each application rate of increasing level of TN from both biosolids. Urea treatments received 50 kg P ha^{-1} by adding superphosphate in the main plots (with size 1.5 m × 12 m). It can be seen that the amount of P added into the second application rate of N (68 kg N ha^{-1}) had 39 kg P ha^{-1} applied which means that it is close to the crop requirement of P (50 kg P ha^{-1}).

7.3.3 Mineralisation of Organic-N

The calculation of mineralisable N in the biosolids was estimated by subtracting the initial available inorganic N from the total mineralised N and represented as a percentage of the organic N. The results are shown in Table 7.10.

Table 7.10 Calculation of the quantity of N (% organic N) mineralised from PAN in two biosolids types to fertiliser

	Clay loam soil		Sandy loam soil	
	ANDB	ADB	ANDB	ADB
N fertiliser equivalency [a]	42	19	40	16
Organic N (% Total N)	98.2	91.5	98.2	91.5
Initial available N (% Total N)	1.8	8.2	1.8	8.2
Mineralisable organic N (% organic N) [b]	41	12	39	9
(EPA, VIC ,2004) assumed values	**15**	**25**	**15**	**25**

[a] Calculation of ryegrass response to applying various rates of N from two different biosolids relative to response to urea (Total-N uptake),

[b] [(PAN – initial available N) / organic N] ×100

These results suggest that in order to achieve the equivalent of 250 kg N ha^{-1} a from urea, a farmer would need to apply 28 t ha^{-1} and 29 t ha^{-1} of the ANDB on the clay loam and sandy soils respectively. Similarly, 112 t ha^{-1} and 149 t ha^{-1} of the ADB would need to be applied to the clay loam and sandy loam soils respectively.

The values derived for the mineralisable organic N are quite different from those assumed by the EPA (EPA, VIC ,2004), but are close to the results obtained by Pu *et al.,* (2008) in Queensland where N mineralisation from anaerobic biosolids on a clay loam soil ranged from 44 to 59 % under a sub-tropical climate. The material also had a similar PAN to values estimated by Gilmour *et al.,* (1999) for anaerobically digested biosolids which was 40 % of the biosolids organic N treated a Captina silt loam (Fine-silty, siliceous, mesic Typic Fragiudult) soil in sorghum sudangrass [Sorghum bicolor]. This value is considerably greater than those usually calculated by the USEPA (1995) for mineralization rate, which emphasizes the necessity to review annual percentages of mineralization rates.

7.4 Implications to the Victorian Guideline for land application of biosolids

The Victorian guidelines for land application of biosolids recommends the use of 15 % and 25 % mineralisation rates of organic N for calculating the amount of PAN from ANDB and ADB respectively (EPA, VIC ,2004). The findings of these field experiments indicate that the more appropriate rate should be 40 % and 11 % ANDB and ADB respectively.

These estimated values for ANDB were significantly higher than the corresponding values recommended by the Victorian guidelines; however, the estimated mineralisation rates of 12 % and 9 % for ADB were significantly lower than the mineralisation rate of 25 % recommended by the Victorian guideline for land application of biosolids.

The method used in this experiment allows the calculation of the mineralisable organic N without the need for a full mass balance experiment, which would normally involve the measurement of microbially sequestered N, plant uptake, NH_3, N_2O, N_2 organic N in the soil and any leached N. The mineralisable organic N is an intrinsic property of the biosolids, which once characterised against a fertiliser such as urea, can used to calculate its behaviour under different environmental conditions,

provided the behaviour of urea is known. This was clearly show by the similarity in values obtained from two different soil types.

7.5 Conclusions

The study has demonstrated that the current estimate of 25 % of the organic N in aerobic biosolids is mineralisable (EPA, VIC ,2004), is an overestimation for the aerobically produced biosolids from BW. In contrast, the 15 % mineralisable organic N expected for anaerobically produced biosolids (EPA, VIC ,2004) is an underestimation for the anaerobically produced biosolids from MRWP. From the results of this study the amount of organic N mineralised is equivalent to between 8 and 9 kg N t^{-1} (28 t ha^{-1} and 29 t ha^{-1}) for ANDB and 2 - 3 kg N t^{-1} (112 t ha^{-1} and 149 t ha^{-1}) for ADB applied in clay loam and sandy loam soil respectively.

This study also showed that the calculated percentage of mineralisable N for either of the biosolids tested was independent of soil type. However, the amount taken up by plants will depend upon soil conditions (fertility, water holding capacity, texture) and climatic conditions (rainfall and temperature). If the relationship between the N uptake from biosolids and the N uptake from urea is known, running a simple experiment with urea can be used to predict the plant available N from the biosolids for a specific crop, under any given soil and environmental conditions. Consequently, once the initial calculation has been done for specific biosolids, it should be unnecessary to repeat the calibration experiments of the type described in this chapter, under multiple soil and climatic conditions. Biosolids generally applied based on N; but, given the amount of P in the biosolids, these application rates may lead to the over application of P with potential undesirable environmental effects. General conclusion of this study will be described in the next chapter (8).

8

8 Conclusion

One of the aims of the laboratory incubation experiment was to determine the rate of mineralisation and the proportion of the organically bound N which was mineralised. This type of experiment has been done by many researchers some of whom say that the results obtained are similar to the results obtained in the field. Others say the results are different. In this study, the results were very different. However, TN was not measured in either the laboratory incubation experiment or the field experiments, so it was not possible to calculate the amount of N lost through denitrification. Since denitification is likely to have been occurring over the whole of the experimental period, even the amount of mineralised N at its maximum value would be less than the true amount. Nevertheless, it was shown that the maximum amount of mineralisation had occurred by day 56 which is consistent with previous research.

The results in the field experiments with and without vegetation gave similar results as shown in Figure 8.1. The measured amount of mineral N was slightly less in the vegetated plots. No measurement was made on the TN in the plants. The results from the field experiment are also compromised because they are based on the assumption that the incorporation depth was 15 cm. If it was less than 15 cm, the estimated % mineralised Organic N would also have been less.

An interesting observation was that the ratio of MBC: MCN in the laboratory incubation experiment started off high, decreased and then increased again at the end. The reverse happened in the field plots. Presumably different microrganisms have specific roles during the experimental period. This needs further investigation.

In both the laboratory and field experiments, ANDB had a greater amount of mineralisable organic N than ADB. The difference was magnified in the sandy loam soil where the values were lower. There was also a difference in the timing of the

maximum amount of mineral N. In the field study this occurred after 42 days. The differences observed between the laboratory incubation experiment and the field plots are not surprising given the difference in aeration, temperature and humidity.

The calibration experiment does not rely on knowing the incorporation depth as both the urea and the biosolids were applied to the soil in the same way. The calculations only require the rate of uptake of N into the plant material with increasing TN applied. This method also accounts for losses due to denitrification and leaching processes since they are the same for both urea and the biosolids. Once the mineralisable organic N for a specific biosolid has been calculated, it is possible to predict its behaviour under different climatic conditions with differing soils provided the behanviour of urea is known.

Recommendations for future work

The results for each of the experiments are shown in Figure 8.1 and they clearly demonstrate that the Victorian Guidelines for the application of biosolids to land need revision.

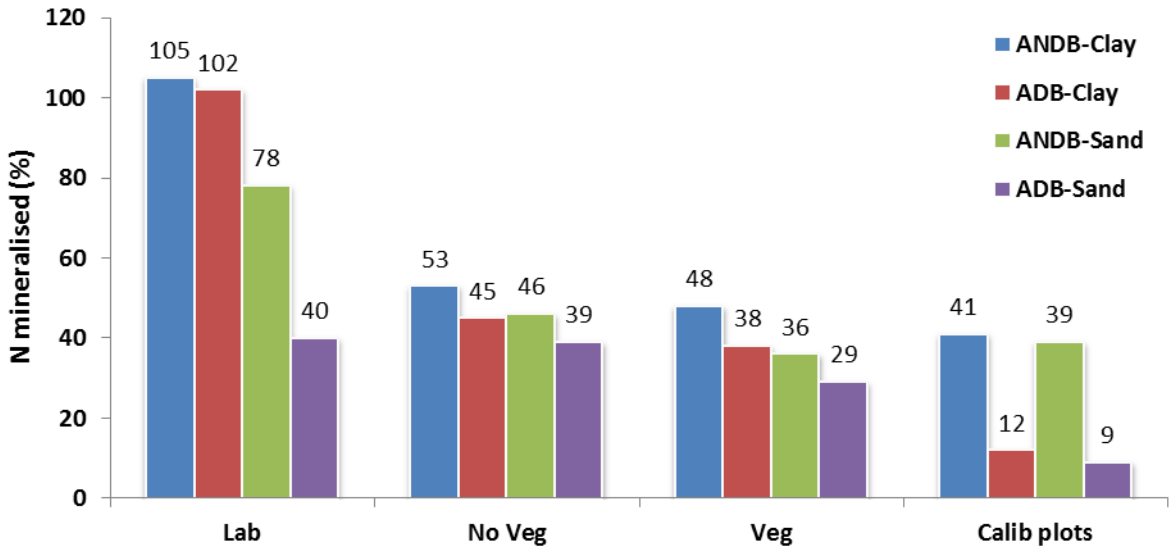

Figure 8.1 Mineralisable N from two biosolids used in this study under laboratory and field experiment

The calibration gave a more reliable estimate of the mineralisable organic N, so this would be the method of choice for farmers wanting to know how much of the biosolids to apply.

A more definitive answer from the laboratory incubation and associated field trial could have been obtained by measuring TN. I would recommend that this be included in any future incubation experiments.

The MBC: MBN ratio provided some interesting results which would be a fertile area for examination. The measurement of MBN and MBC relied upon values obtained from the literature for the fumigation and extraction efficiency. I would recommend that this should be tested prior to starting any future experiments.

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Appendices

A. Biosolids N data collected from the published and grey literature

Table A.1 Total and mineral (NH_4-N and NO_3-N) values reported for different biosolids types reported in the published and grey literature

Biosolids type	Total N (%) mean	Total N (%) median	NH_4-N (mg kg^{-1}) mean	NH_4-N (mg kg^{-1}) median	NO_3-N (mg kg^{-1}) mean	NO_3-N (mg kg^{-1}) median	n	Reference
Aerobic	5.4						1	Heukelekian and Rudolfs (1928)
Aerobic	2.7		1360.0		44		1	Parker and Sommers (1983)
Aerobic	4.9(0-5-7.6)		950.0		300(7-830)	180(7-830)	38 (total N), 33 (NH_4-N), 8 (NO_3-N)	Sommers (1977)
Aerobic	2.9(1.5-4.7)	2.7	987.8 (102-2378)	1172.0	53.3		6	Serna and Pomares (1992)
Aerobic	5.4		241.0					Hseu and Huang (2005)
Aerobic	2.7		1800.0					Bozkurt et al. (2006)
Aerobic liquid	6.8		884				1	Magdoff and Chromec (1977)
Aerobic liquid	4.8							Sigua et al. (2005)

Biosolids type	Total N (%) mean	Total N (%) median	NH_4-N (mg kg^{-1}) mean	NH_4-N (mg kg^{-1}) median	NO_3-N (mg kg^{-1}) mean	NO_3-N (mg kg^{-1}) median	n	Reference
Aerobic liquid	4.8							Adjei and Rechcigl (2002)
Aerobic, ferric chloride, alum, dewatered	4.6 (4.3-4.9)						6	Esteller et al. (2009)
Aerobic, dewatered	4.0		7900.0		0.3			Eldridge et al. (2008)
Aerobic, dewatered	6.1		2460.0					Robinson and Polglase (2000)
Aerobic, dewatered	6.2		2800.0		2		3	Pu et al. (2008)
Aerobic, dewatered	5.8		11200.0					Petersen (2003)
Aerobic, dewatered	5.6		2810.0		41		13	Barry et al (2006)
Aerobic, belt-pressed	7.3		6700.0					O'Shaughnessy et al. (2009)

Table A.2 Nitrogen mineralisation rates through laboratory and field investigations and experimental conditions reported in the literature

Biosolids type	Application rate	N min (% org-N) Mean (range)	Soil pH	Soil type/ texture	Temp (ºC)	Moisture	Duration (days)	Method	n	Reference
Aerobic	150-900 mg N kg^{-1}	50.5(36.1-60.8)		fine sandy loam	17		91	leached	3 rates, 2 reps	Magdoff and Chromec (1977)
Aerobic	13.4 g kg^{-1}	28.5(25-32)	5.9	silt loam	23	0.3 bar	112	soil incubation (leached & non-leached)	2 methods, 2 reps	Parker and Sommers (1983)
Aerobic	28 g kg^{-1}	34.3(24-40)			25	66	112	non-leached, aerobic	6 sludges, 3 reps	Serna and Pomares (1992)
Aerobic		5(3-7)	6.8	sandy loam		40	42	non-leached	2 sludges, 3 reps	Iakimenko et al. (1996)
Aerobic, liquid		54.0	6.1/ 7.1	sandy loam/loam	25	Field moist	119	leached	4 rates, 4 reps, 2 soils	Magdoff and Amadon (1980)
Aerobic, sand-bed dried	8.25-33 kg ha^{-1}	49(19-107)	7.8	sandy loam/sandy clay loam	30	34kPa	480	lab incubation (leached/non-leached)	4 rates, 2 soils, 2 methods, 2 reps	Garau et al. (1986)
Aerobic, partially dewatered	12-54 t ha^{-1}	52.1(45.3-58.9)		alluvial clay loam	15.7-29.4		231	N budget in a forage system	2 rates, 3 reps	Pu et al. (2008)

Biosolids type	Application rate	N min (% org-N) Mean (range)	Soil pH	Soil type/ texture	Temp (ºC)	Moisture	Duration (days)	Method	n	Reference
Aerobic, dewatered	22 t ha^{-1}	34.0		silty clay loam	11-22	1237 mm rainfall		Turf uptake of N, biosolids incorporated	4 reps	Eldridge et al. (2008)
Aerobic, dewatered	23 t ha^{-1}	53.0		silty clay loam	11-22	665 mm rainfall	365	Field incubation	1 rate, 4 reps	Eldridge et al. (2008)
Aerobic, dewatered	6-110 t ha^{-1}	57		Range of clay soils			~365	Partial N budget, range of crops	6 rates, 3 reps	Barry et al. (2006)
Anaerobic	150-900 mg N kg^{-1}	20.8(13.7-25.2)		fine sandy loam	17		91	Lab incubation, leached	3 rates, 2 sludges	Magdoff and Chromec. (1977)
Anaerobic	13.4 g kg^{-1}	15.3(4.0-31.0)	5.9	silt loam	23	0.3 bar	112	Soil incubation, leached, sludges applied air-dried and ground	11 sludges, 2 methods, 2 reps	Parker and Sommers (1983)
Anaerobic dewatered		25.0								Smith et al. (1998a)
Anaerobic, liquid	6.7 t ha^{-1}	31(28-34)		silt loam	25	40	75	lab incubation, non-leached	2 sludges, 3 reps	Gilmour et al. (2003)
Anaerobic, dewatered		35.2(31.9-38.5)						est. from relative PAN	4 reps, 2 years	Gilmour and Skinner (1999)

Biosolids type	Application rate	N min (% org-N) Mean (range)	Soil pH	Soil type/ texture	Temp (ºC)	Moisture	Duration (days)	Method	n	Reference
Anaerobic, dewatered	10 g kg^{-1}	32(27-37)					168	Lab incubation, non-leached, two soil, range of pH, moisture & temp	4 reps	Terry et al. (1981)
Anaerobic, dewatered		29.3(26.5-32.1)						Estimated from crop uptake trial	5 rates, 4 reps, 2 sites,	Barbarick and Ippoito (2000)
Anaerobic, dewatered	16-72 t ha^{-1}	45.4(42.6-48.1)		alluvial clay loam	15.7-29.4	471 mm rainfall	231	N budget in a forage system	2 rates, 3 reps	Pu et al. (2008)
Anaerobic, dewatered	156-1172 kg N ha^{-1}	33.1(24.9-38.7)	4.2	heavy clay	22.1-31.6	irrigated daily	121	Field incubation	4 rates, 3 reps	Sripanomtanakorn and Polprasert (2002)
Anaerobic, dewatered		43.5(30-57)		silt loam	25	Field capacity	252	Soil incubation, non leached	2 sludges, 4 reps	Adegbidi et al. (2003)
Anaerobic, dewatered	0-13.3 t ha^{-1}	17.3(6.8-28)		loamy sand			3 years	Field trial		Morris et al. (2003)
Anaerobic, dewatered	400 mg N kg^{-1}	15.2(5.9-29)	5.4/4.5	sandy/ stoney silt loam	10-20	Field capacity		Lab incubation, leached	2 soils, 3 reps	Wang et al. (2003)

Biosolids type	Application rate	N min (% org-N) Mean (range)	Soil pH	Soil type/ texture	Temp (ºC)	Moisture	Duration (days)	Method	n	Reference
Anaerobic, dewatered	8-112 t ha^{-1}	60		Range of clay soils			~365		6 rates, 3 reps	Barry et al. (2006)
Anaerobic, dewatered	20 g kg^{-1}	90.0		loamy sand	21		90	soil column, leached	3 reps	Mendoza et al. (2006)
Anaerobic, dewatered	10 t ha^{-1}	17.3 (14.2-20.4)	8.2/ 6.8	silty clay/sandy silt loam	10-30	15-22%	90	Field investigation, no crop	2 soils, 3 reps	Rigby et al. (2009)
Anaerobic, dewatered	0-15 t ha^{-1}	38.1	6	sand			123	Field trial, estimated from relative response	3 reps	Rigby et al. (2010)
Anaerobic, dewatered	7.5 t ha^{-1}	35.0		brown Sodosol	12.5	70	56	soil incubation, non-leached	3 reps	Ives et al. (2010)
Anaerobic, thermal hydrolysis, dewatered	0-13.3 t ha^{-1}	16.2 (14.8-16.2)		sandy loam			3 years	Field trial	3 reps, 3 years	Morris et al. (2003)
Composted	8 t ha^{-1}	24.5 (10.9-38.1)		sandy/clayed	25	Field capacity	161	soil incubation, non-leached	2 soils, 2 reps	Correa et al. (2006)
Raw, lime, compost		-10 (-15 – -5)	5.6	silty clay loam			168	Pot trial. Tall fescue	2 sludges, 4 reps	Bowden et al. (2007)

Biosolids type	Application rate	N min (% org-N) Mean (range)	Soil pH	Soil type/ texture	Temp (ºC)	Moisture	Duration (days)	Method	n	Reference
Wet-air oxidised	13.4 g kg^{-1}	1.5 (0-3)	5.9	silt loam	23	0.3 bar	112	soil incubation, leached, sludges applied air-dried and ground	2 sludges, 2 reps	Parker and Sommers (1983)
Wet-air oxidised	13.4 g kg^{-1}	35.8 (11-58)	5.9	silt loam	23	0.3 bar	112	soil incubation, non-leached, applied air-dried/ ground	4 sludges, 2 methods, 2 reps	Parker and Sommers (1983)
Raw, dewatered	10 t ha^{-1}	19.8 (18.2-21.3)	8.2/ 6.8	silty clay/sandy silt loam	10-30	15-30%	90	Field experiment, no crop	2 soils, 3 reps	Rigby et al. (2009)
Activated sludge		60.5 (59-62)		sandy loam	22	Field capacity	126	lab incubation, non-leached	2 methods of incorporation, 4 reps	King (1973)
Raw primary, air-dried	50 t ha^{-1}	48.4 (36-58)			30		182	soil incubation, leached	5 soils, 2 reps	Chae et al. (1986)

Biosolids type	Application rate	N min (% org-N) Mean (range)	Soil pH	Soil type/ texture	Temp (ºC)	Moisture	Duration (days)	Method	n	Reference
Activated, air dried	6.7 t ha^{-1}	19.0		sandy loam	25	40	75		3 reps	Gilmour et al. (2003)
Activated sludge, lagoon dewatered	6.7 t ha^{-1}	0.0		sandy loam	25	40	75		3 reps	Gilmour et al. (2003)
Waste activated, alum dosed, dewatered	0-15 t ha^{-1}	64.1	5	sand			123	Estimated from crop uptake relative to urea	3	Rigby et al. (2010)